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Early View

Review

Smoking and vaping alter genes related to mechanisms of SARS-CoV-2 susceptibility and severity

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TITLE: Smoking and vaping alter genes related to mechanisms of SARS-CoV-2 susceptibility and severity

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Take home message:

Cigarette smoke and electronic cigarettes alter genes and subsequent pathways of interest involved in SARS-CoV-2 entry and hyperinflammatory immune responses. Direct implications on risk of infection and disease severity requires more research.

Key words:

smoking smoking and health viral respiratory infection

ABSTRACT

Evidence for the impact of smoking on COVID-19 is contradictory, and there is little research on vaping. Here we provide greater clarity on mechanisms perturbed by tobacco cigarette, electronic cigarette and nicotine exposures that may impact the risks of infection and/or disease severity.

Following PRISMA guidelines, OVID and Web of Science databases were searched. Study design and exposure-induced gene expression changes were extracted. Each study was quality assessed and higher confidence scores were assigned to genes consistently changed across multiple studies following the same exposure. These genes were used to explore pathways significantly altered following exposure.

125 studies provided data on 480 genes altered by exposure to tobacco cigarettes, e-cigarettes, nicotine or SARS-CoV-2. Genes involved in both SARS-CoV-2 viral-entry and inflammation were changed following exposure. Pathway analysis revealed that many of those genes with high confidence scores are involved in common cellular processes relating to hyperinflammatory immune responses.

Exposure to tobacco cigarettes, e-cigarettes, or nicotine, may therefore impact initial host-pathogen interactions and disease severity. Smokers and vapers of e-cigarettes with nicotine, could potentially be at increased risk of SARS-CoV-2 infection, associated cytokine storm, and acute respiratory distress syndrome. However, further research is required, particularly on e-cigarettes, to determine the biological mechanisms involved in perturbation of viral-entry genes and host-pathogen interactions and subsequent responses within the respiratory tract. This will improve our physiological understanding of the impact of smoking and vaping on COVID-19, informing public health advice and providing improved guidance for management of SARS-CoV-2 and other respiratory viruses.

1.0 INTRODUCTION

The global COVID-19 pandemic and its causative pathogen, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has been responsible for millions of mortalities worldwide [1]. SARS-CoV-2 continues to transmit through the population and poses a threat to public health globally. Host cell entry is predominantly through the angiotensin converting enzyme 2 (ACE2) receptor, which is a part of the renin angiotensin system (RAS) [2, 3]. Entry is further enhanced with the priming of the virus' spike proteins by host cell enzymes transmembrane serine protease 2 (TMPRSS2), furin, Neuropilin-1 (NRP1) receptor, CD147 receptor and/or cathepsins [4-10]. Following the initial infection, COVID-19 symptoms are flu-like with severe cases involving a hyperinflammatory response that can result in a cytokine storm and further complications such as acute respiratory distress syndrome (ARDS) and cardiac failure [11]. While vaccines are widely available and act to reduce severe disease, it is important to identify specific at-risk populations to ensure there is targeted public advice.

Within the literature, the epidemiological associations between smoking and COVID-19 appear contradictory. Some studies report that current smokers have a reduced risk of SARS-CoV-2 infection, while others suggest that current smokers have a higher risk of COVID-19 hospitalisation than former or never smokers [12]. Initial epidemiological investigations using hospital records reported that many smokers required aggressive interventions and ventilation [13], but when adjusting for comorbidities these outcomes became non-significant in other studies [14, 15]. This is likely due to the many comorbidities associated with smoking, highlighting the difficulty of distinguishing the impact of smoking alone on COVID-19 and the need for mechanistic studies to underpin the biological plausibility of epidemiological associations. Current mechanistic evidence is largely centred around ACE2, nicotinic acetylcholine receptors (nAChRs) and RAS, with potential crosstalk between ACE2 and nAChRs via RAS implicated in both reduction of SARS-CoV-2 infection [12, 16, 17] and more severe COVID-19 through stimulation of inflammatory signalling pathways [18-20]. This suggests complex mechanisms that are dependent on infection/disease stage.

E-cigarette use in the UK is increasing as many current smokers use vaping as a tool to stop smoking. Many ex-smokers continue to vape and the number of never smokers that have begun vaping is increasing [21]. Despite this, there is little research on the susceptibility of e-cigarette users to COVID-19. Initial indications suggest they may have an increased risk of SARS-CoV-2 infection, but this may differ with nicotine content, flavours and propylene glycol: vegetable glycerine (PG:VG) content [22, 23].

The aims of this review were therefore to: 1) identify key genes and pathways of interest altered by tobacco cigarette, e-cigarette, or nicotine exposures that may affect viral-entry (and therefore the risk of an individual to SARS-CoV-2 infection) and associated disease severity; and 2) perform a weight-of-evidence based meta-analysis of key mechanistic studies to clarify the existing contradictory literature on the potential impacts of smoking and vaping on COVID-19.

2.0 MATERIALS & METHODS

2.1 Search strategy

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, a thorough search of the literature, using OVID and Web of Science databases, was undertaken (up to November 2022). Within each database, two individual searches were carried out, one related to smoking exposure (tobacco cigarette smoke/tobacco cigarette smoke condensate

(herein referred to as cigarette smoke (CS)), vaping (e-liquid, e-liquid condensate e-cigarette vape (herein referred to as e-cigarettes), or nicotine), pathways of interest and the respiratory tract; and a second focussed on the interaction of respiratory viruses (with a focus on SARS-CoV-2), smoking exposure and pathways of interest (detailed in S1). Additional search terms related to heated tobacco products were considered but did not provide any further results eligible for inclusion. Results were collated and duplicates removed.

2.2 Eligibility and exclusion criteria

Studies were screened for eligibility by their title, abstract and full text. RB screened all studies, with 10% of the total screened by EM and AB. Discrepancies were discussed and a consensus decision was made. Inclusion and exclusion criteria (Table 1) were developed to identify key pathways altered following CS, e-cigarette or nicotine exposure in respiratory epithelial cells that may affect the risk of a normal, healthy individual to SARS-Cov-2 infection and/or COVID-19 severity. Thus, we were interested in the impact of smoking/vaping/nicotine on healthy individuals only and not those with other respiratory pathologies or non-viral respiratory infections.

2.3 Data extraction

Data from eligible studies were extracted manually and collated (RB). Extracted data included: details of exposure (type, dose and time, brand), model (species, cell type) and findings (mRNA and protein) (detailed in S2). Where supplementary information was available, the data for the top ten upregulated and/or downregulated genes were extracted.

2.4 Quality assessment

A quality-scoring tool was adapted from previous reviews [24, 25]. Included studies were assessed against 6 domains designed to measure their ability to address the review aim. These were cell model, route of exposure, dose, gene expression, cytotoxicity and SARS-CoV-2 challenge (detailed in S3). Each study was given a score for each domain, higher scores were assigned for:

- Physiologically-relevant models such as primary epithelial air-liquid-interface (ALI) cultures *in-vitro* and non-human primates *in-vivo*
- Physiologically-relevant routes of exposure such as aerosol systems *in-vitro* and intranasal administration *in-vivo*
- Human exposure relevant doses with appropriate controls or dose-dependent responses
- Validation of gene expression changes with knockout, silencing, inhibitor, or agonist investigations
- Use of multiple cytotoxicity assays (oxidative stress, DNA damage, barrier integrity etc)
- SARS-CoV-2 challenge with wild-type infection.

Domain scores were combined and averaged to form an overall quality rating of very low, low, medium or high for each study.

2.5 Meta- and pathway-analysis

A weight of evidence approach was developed to generate a confidence score (for the mRNA and/or protein) that combined study frequency (the number of studies investigating the gene of interest (GOI)), consistency (the overall level of change of the GOI across all studies) and quality (the overall quality score of the studies from which each GOI was extracted). The frequency of all upregulated GOI extracted from high-quality studies were multiplied by 2, medium-quality by 1.5, low-quality by 1 and

very low-quality by 0.5; while the frequency of all downregulated GOI extracted from high-quality studies were multiplied by -2, medium-quality by -1.5, low-quality by -1 and very low-quality by -0.5. Values for each GOI within each exposure type were combined to generate overall confidence scores for the mRNA and protein per GOI per exposure. Note that e-cigarette data was subdivided into those with nicotine (EC+N) or without nicotine (EC-N). The higher the positive confidence score, the more robust the evidence for upregulation and the greater the negative confidence score, the more robust the evidence for downregulation. Ensembl was used to convert any non-human GOI into their human homologues prior to pathway analysis.

To simplify further for pathway analysis, mRNA and protein confidence scores for GOI were combined for each exposure. GOI with the 10 greatest upregulated and downregulated confidence scores for each exposure type (where data was available) were imported into Cytoscape (version 3.10.0) for pathway analysis using the KEGG database (version 22 MAY 2022). Overall scores for pathways identified as significant or as having a large proportion of high confidence GOI present were generated by combining the confidence scores of all extracted genes for every exposure (where data was available).

3.0 RESULTS

3.1 Overview of search results

Of the 7,808 records identified by the literature search, 125 were selected for inclusion and categorised by exposure type (Figure 1A). Many excluded studies focussed on comorbidities rather than investigating the response within a healthy respiratory system. Within the included studies, most investigated the epithelial cell response to CS (n=87, 70%) with fewer on either e-cigarette, nicotine or SARS-CoV-2 exposures (n=9, 7%; n=14, 11%; n=7, 5%; respectively) or multiple exposure types (n=8, 5%). The majority of included studies were performed *in-vitro* (n=78, 62%) *vs in-vivo* (n=22, 18%), while 25 (20%) used both *in-vitro* and *in-vivo* models. Most *in-vitro* studies used human epithelial cell lines or primary cells (n= 71, 57%), with 31 (25%) using other species (including mouse, rat, ferret, guinea pig, sheep and non-human primates) and 23 (18%) using human plus another species. Throughout the included studies, models, routes of exposure, doses, end-point assays, GOI, cytotoxicity and/or viral challenge varied, making direct comparisons across the included studies difficult.

3.2 Quality assessment

Quality scores per domain per study are detailed in Supplementary Information S4. The majority of the included studies were high- or medium- quality overall (67%, (n=84), with only 6% (n=8) of very low-quality (Figure 1B). Of the *in-vitro* studies, 28 (27%) used high-quality cell models such as primary cells cultured at ALI for any length of time, or cell lines cultured at ALI for 14 days or more allowing the cells to differentiate into a pseudostratified epithelium. The remaining *in-vitro* studies used submerged monolayer cell culture or cell line ALI cultures for less than 14 days, which may therefore not be fully differentiated. The route of exposure for *in-vitro* studies was predominantly via direct application of a solution containing tobacco cigarette condensate, e-liquid or nicotine (n=94, 91%) *vs* aerosolised delivery (n = 9). Likewise, almost all *in-vivo* studies used a low-quality model (n=46, 98%) combined with a low-quality route of exposure (n=37, 81%). Only 16 included studies (12%) directly measured the effect of CS, e-cigarettes or nicotine on infection using wild-type or pseudo-SARS-CoV-2. However, 90% of the studies (n=112) performed some additional investigation or validation of exposure-induced gene expression changes.

3.3 Meta-analysis

3.3.1 GOI

While included studies provided data on exposure-induced expression changes in 480 genes (detailed in S5), the vast majority (n=351, 73%) were only measured in one paper and were thus assigned a single confidence score (Figure 2A). Of those that were assigned two confidence scores (n=82, 17%), many were only measured in one exposure type, predominantly CS. Despite obvious data gaps and variations in confidence across exposures, some exposure-induced trends were identified.

Those genes with 5 or more confidence scores are shown in Figure 2B. These GOI have the most data available to compare across different types of exposures. Many of these genes (ACE2, AKT1, CHRNA5, CXCL8, IL6, MMP9, NFKB1, TMPRSS2, TNF) were upregulated by CS. In contrast, e-cigarettes downregulated CDH1, CHRNA5, IL6 and TNF. E-cigarette-induced changes in ACE2, TMPRSS2 and MM9 were less clear, with some contrasting evidence depending on the presence of nicotine (upregulated with nicotine and downregulated without). Exposure to nicotine alone followed a similar trend to CS with upregulation of ACE2, AKT1, CHRNA5, CXCL8, IL6, NFKB1, TMPRSS2 and TNF, and downregulation of CDH1. EC+N also downregulated CDH1, and exposure to SARS-CoV-2 induced upregulation of ACE2, CXCL8, IL6 and TNF.

Exposure-induced changes in additional nAChRs and SARS-CoV-2 viral-entry genes are shown in Figures 2C-D. The data on nAChRs predominantly comes from exposures to nicotine alone, which upregulated CHRNA1-7 and CHRNB2&4. There was also some evidence for CS-induced upregulation of CHRNA1/3/5/7 and downregulation of CHRNA6/B4, with contrasting results For CHRNB2/G. The data on other viral-entry genes comes from exposures to CS, which provided some evidence for upregulation of BSG, CTSB, FURIN and TMPRSS4, and downregulation of CTSL and NRP1.

3.3.1 Pathway analysis

Significantly altered pathways following CS, e-cigarette or nicotine exposures included AGE-RAGE (in diabetic complications), IL17 and VEGF signalling, with links to other diseases/infections such as Chagas disease, influenza A, human cytomegalovirus and Karposi sarcoma-associated herpesvirus (Figure 3A). Examination of the confidence scores of the gene expression changes behind these pathways (Figures 3B-D) demonstrated that CS upregulated many of the genes in the AGE-RAGE (in diabetic complications), IL17 and VEGF signalling pathways. While data for these genes following SARS-CoV-2 and e-cigarette exposures (particularly EC-N) was limited, there was some evidence that, in contrast to CS, EC-N and EC+N downregulated GOI within the same 3 pathways. Nicotine- or SARS-CoV-2-incuded GOI changes more closely resembled those following CS exposure.

In summary whilst data was limited for the impact of EC-N, genes and pathways of interest identified in this review were altered following exposure to CS, EC+N, or nicotine alone, some of which were similarly altered by SARS-CoV-2 infection.

4.0 Discussion

Data was extracted from 125 studies identifying genes and pathways perturbed by CS, e-cigarette, nicotine alone or SARS-CoV-2 infection to investigate the potential impact of smoking/vaping/nicotine on the risk of SARS-CoV-2 infection and disease severity. This identified potential biological mechanisms for further investigation, but also highlighted knowledge gaps and factors to consider when collating and interpreting evidence.

4.1 Study design

Many studies used either cell donors with comorbidities, or models (*in-vivo* and *in-vitro*) that scored as low quality because they lacked physiological relevance. More physiologically-relevant *in-vitro* cell models include microfluidic systems, 3D co-cultures and primary human epithelial cells cultured at an ALI for a suitable length of time, which allows differentiation of basal cells into ciliated or mucus producing goblet cells with tight junctions that form an epithelial barrier [26, 27]. Cells cultured at ALI have their apical surface in contact with air, enabling aerosol exposures. ALI combined with aerosol exposures have greater physiological-relevance compared to submerged cultures (test substance added into the media covering cell monolayer) or suspension exposures (test substance added in solution to ALI culture). Cellular responses following aerosol exposure, including the release of cytokines, are more likely indicative of the aerosol constituents, rather than the result of stress from the abnormal environment within submerged cultures or suspension exposures. Nevertheless, more physiologically-relevant models are time-consuming, expensive and can create large data variability, especially when using primary cells from multiple donors. Cell lines differentiated at ALI are an alternative, less variable, option. Full validation is essential, however, to fully characterise physiological relevance and presence of key mechanisms [26, 27]. Inconsistencies within study models, exposure methods and doses, made comparing overall outcomes challenging.

Extracting mRNA and protein data also revealed inconsistencies. Much of the data available following exposure provided information on either mRNA or protein levels alone, creating gaps in the dataset. There was little weight of evidence as many GOI were only reported in one study. Only ACE2 expression data was available following all exposures. Studies on e-cigarettes were particularly lacking, with the impacts of different PG:VG content, flavours and nicotine composition remaining largely unstudied [21, 28].

4.2 Genes of interest

GOI were selected as either: 1) having key roles in SARS-CoV-2 viral-entry (ACE2, TMPRSS2, TMPRSS4, NRP1, BSG, FURIN, CTSL, CTSB), 2) potentially explaining contradictory results/findings (nAChRs and relevant subunits), and/or 3) within the top 10 genes with the greatest coverage across the different exposure types (ACE2, AKT1, CDH1, CHRNA5, CXCL8, IL6, MMP9, NFKB1, TMPRSS2, TNF). Key GOI are discussed below (and summarised in Figure 4) with respect to potential impacts on risk of SARS-CoV-2 infection and COVID-19 severity.

4.2.1 Risk of infection

The roles of ACE2 and TMPRSS2 in SARS-CoV-2 viral-entry are well reported and TMPRSS4, NRP1, BSG (also known as CD147), FURIN, CTSL and CTSB also assist with viral-entry [29]. ACE2 was upregulated by SARS-CoV-2 infection [30-33] and ACE2 plus many of these other viral-entry GOI were consistently upregulated by CS [23, 30, 32, 34-44], with only three contradictory studies reporting ACE2 [35, 45] and NRP1 [46] downregulation. Significantly increased SARS-CoV-2 or pseudoviral infection rates were also measured in nasal epithelial cells from smokers [30] and primary human bronchial epithelial cells cultured at ALI [47], compared to controls. Hence, smokers may be at greater risk of infection with SARS-CoV-2 than non-smokers.

Exposure to EC+N generally upregulated ACE2 [23, 48-50] and TMPRSS2 [50]. One study reported downregulation in ACE2 [22], with more evidence of downregulation induced by EC-N [49, 50]. This

suggests that nicotine plays a key role in the impact of e-cigarettes on viral uptake, potentially increasing the risk of SARS-CoV-2 infection in vapers *vs* non-vapers.

Nicotine-enhanced viral uptake is suggested to involve nAChRs. The α7 [38, 46, 51, 52] nAChR and α5 [53] α3 [54] and α1 [53] related subunits, were upregulated following exposure to CS, with limited data following e-cigarette exposure. Nicotine-induced upregulation of ACE2 was mediated by α7 nAChR in mice [49] and human bronchial epithelial cells grown in monolayer exposed to nicotine at a dose equivalent to smoking one cigarette [55]. Both studies validated findings, using α7 nAChR knockout experiments or gene silencing. The nicotine-derived nitrosamine ketone within tobacco smoke also upregulated α7 nAChR, increasing the sensitivity of small bronchial epithelial cells to stresses [46]. Wider literature suggests that in response to stress, ACE2 levels increase, as part of RAS, to elicit stress-dampening actions [56] with the promotion of oxidative stress in ACE2 knockout mice [57]. Stimulation of α7 nAChR following exposure to nicotine may increase ACE2 as demonstrated in bronchial epithelial cells [38]. CS and e-cigarettes containing nicotine may therefore promote α7 nAChR-mediated upregulation of ACE2, also potentially increasing the risk of SARS-CoV-2 infection in smokers and vapers *vs* non-smokers/vapers.

Some studies also reported altered expression of key viral-entry genes within different regions of the respiratory tract and between different sexes. ACE2 was upregulated in bronchial cells but downregulated in alveolar cells [36]; and, while differences in ACE2 expression were not significantly different in smokers across nasal, bronchial and alveolar tissue, non-smokers had significantly higher ACE2 expression in alveolar compared to their nasal and bronchial regions ($p=0.039$; $p=0.007$, respectively) [45]. In addition, greater expression of ACE2 was observed in the goblet cells of smokers, and club cells of non-smokers [41]. With respect to sex differences, one study reported an e-cigarette-induced upregulation of ACE2 mRNA expression in males only [48]; and another observed that, despite a greater ACE2 protein abundance in females, only male ACE2 protein abundance was reduced following PG exposure [49]. Androgen signalling may contribute to these differences since increased androgens in smokers were implicated in the increased expression of both TMPRSS2 and ACE2 [40]. Hence, cell types, respiratory tract region, sex and smoking status can all influence ACE2 expression, which may contribute to the conflicted literature surrounding smoking/vaping and risk of infection with SARS-CoV-2.

4.2.2 COVID-19 severity

AKT1 is associated with viral replication [58] and knockdown of AKT or silencing/inhibition of P13K/Akt/mTOR pathways inhibits the replication of respiratory infections such as influenza A [59] and Middle East respiratory syndrome coronavirus (MERS-CoV) [58, 60]. Influenza A and MERS-CoV share transmission and genetic similarities with SARS-CoV-2, respectively [61], so exposure-induced changes to AKT1 expression could impact SARS-CoV-2 replication. AKT1 was upregulated with high confidence by CS [51, 62-64] or nicotine alone [51, 55, 65]. This may counteract the viral-induced reduction of AKT reported in one study [66], increasing SARS-CoV-2 replication following infection and the subsequent risk of severe disease in smokers.

Many GOI relate to the pro-inflammatory immune response. Pro-inflammatory cytokines IL-6 and TNF, chemokine CXCL8 and/or the NFKB1 inflammatory subunit were upregulated following exposure to CS [18, 35, 64, 67-87], nicotine alone [51, 88-90] or SARS-CoV-2 [32, 35, 66, 91]. While inflammation is a key part of the beneficial immune response, hyperinflammation can be detrimental and the drivers of the switch from beneficial to detrimental remain unknown. An elevated IL-6 serum concentration

is observed in patients with COVID-19 and is strongly associated with adverse clinical outcomes, suggesting it is a predictor of/linked to more severe disease [92, 93]. TNF and NFKB1 are involved in the cytokine storm and a hyperinflammatory state, and increased levels are indicative of severe COVID-19 [93, 94]. CXCL8 elevation is a prognostic marker for those at a high risk of ARDS and of patients at a high risk of experiencing severe COVID-19 [95, 96]. The induction of a pro-inflammatory environment in smokers may therefore contribute to, and exacerbate, a cytokine storm, leading to more severe COVID-19 and ARDS [93]. There was some limited evidence of e-cigarette-induced IL6 and TNF downregulation, and NFKB1 upregulation [22, 49], suggesting that vapers may be at a lower risk of developing severe COVID-19 *vs* smokers but at increased risk *vs* non-smokers.

MMP9 is a matrix metallopeptidase elevated in the plasma of patients with severe COVID-19 and correlated with in-hospital deaths [97]. Upregulation of MMP9 by CS [70, 74, 98-101] and EC+N [49] suggests an increased risk of developing severe COVID-19. The downregulation of MMP9 following exposure to EC-N [49] implies no additional risk for those using nicotine-free e-cigarettes and indicates a role for nicotine in MMP9 expression. The latter is supported by α7 nAChR-mediated upregulation of MMP9 [102], highlighting another potential role for nicotine and nAChRs in COVID-19 severity in addition to increased infection risk (section 4.2.1).

ACE2 is also part of RAS, which despite originally being identified as the pathway regulating blood pressure, has more recently been shown to play a key role in inflammation [2, 56]. Within RAS, ACE2 and its homologue ACE balance anti- and pro-inflammatory responses, respectively [103]. It is widely reported that the process of SARS-CoV-2 uptake ultimately downregulates ACE2 expression [2]. Thus, while the different roles of ACE2 as the key viral uptake receptor (increasing risk of infection) and mediator of anti-inflammatory responses (protecting against disease) appear contradictory, they should not be considered distinct. Following an initial increase in ACE2-mediated viral uptake, the levels of ACE2 fall, tipping the balance towards ACE and a more pro-inflammatory environment [103]. Thus, the potential impact of smoking/vaping on severe disease through modification of ACE2 is complex and depends on the specific part of the disease process being measured. This is likely a major contributor to the contradictory literature.

Gender differences in SARS-CoV-2 infection risk, may also impact COVID-19 severity. In the wider literature, males are frequently reported as having higher rates of COVID-19 mortality and severe disease compared to females [104, 105]. The most plausible explanation for this is gender disparity in hormone levels and immune responses. Estrogen in females is considered to help modulate the immune system and provide additional protection from severe inflammation [104-106], whereas androgens in males are associated with over-active immune cells and exacerbation of inflammation and disease severity [106]. The latter, in combination with an elevated inflammatory response following exposure to CS or EC+N, may lead to more severe COVID-19 in male smokers.

4.3 Pathways of interest

Pathways of interest were selected as 1) the most significantly enriched (IL-17 signalling and AGE-RAGE signalling pathway in diabetic complications) or 2) significantly enriched with wider literature supporting a potential role (VEGF signalling). Key pathways are discussed below (and summarised in Figure 4) with respect to potential impacts on risk of SARS-CoV-2 infection and COVID-19 severity.

4.3.1 Risk of infection

VEGF signalling drives angiogenesis by inducing cell survival, proliferation and endothelial migration. Most genes involved in VEGF signalling were upregulated following exposure to either CS or nicotine alone [46, 52, 70], including its activator VEGFA [70]. VEGFA is able to activate the VEGF signalling cascade by binding to VEGF [107, 108]. VEGFA also shares a common binding pocket (b12b domain) on the viral-entry receptor NRP1 [10, 109] and therefore may alter SARS-CoV-2 uptake. Upregulation of VEGFA following CS or nicotine exposure [70], could compete with the SARS-CoV-2 spike protein for the NRP1 binding pocket. This highlights the complexity of viral uptake and the need to understand the affinity of SARS-CoV-2 for, and expression levels of, different receptors. While smokers may have less risk of viral-entry via NRP1 compared to non-smokers, SARS-CoV-2 would still be able to enter cells via other genes and proteases (such as TMPRSS2 and ACE2), which were upregulated by CS, EC+N or nicotine alone.

4.3.2 COVID-19 severity

VEGF signalling may also impact COVID-19 severity. SARS-CoV-2 binding to NRP1 can block VEGF-related signalling, which reduces pain perception [109]. Increased VEGFA is associated with inflammatory-related chronic pain in a variety of conditions [110, 111] and substantially lower levels of VEGFA are reported in the sera of asymptomatic compared to symptomatic COVID-19 patients [112]. Thus, smoking or nicotine induced up-regulation of VEGFA and VEGF signalling may lead to greater symptomatic disease.

AGE-RAGE signalling can disrupt the extracellular matrix, enhancing oxidative stress, and stimulating NFKB signalling [113]. NFKB1 is a signalling molecule within the AGE-RAGE pathway and, as previously described in section 4.2.2, increased levels can be used as a prognostic indicator of severe COVID-19 [94]. AGE-RAGE has been widely studied and implicated in diabetic complications [114] and hyperactive AGE-RAGE signalling in such comorbidities is already considered a risk factor for severe COVID-19 [115, 116]. Exposure to CS or nicotine upregulated many genes within AGE-RAGE signalling with high confidence, including CCND1, CXCL8, HRAS, IL6, KRAS, and TNF [18, 22, 32, 64-76, 78-81, 83, 89, 90, 117-121], suggesting that smokers have a hyperactive AGE-RAGE and are therefore more at risk of severe COVID-19. In contrast, the limited data available suggested EC+N and EC-N do not upregulate the AGE-RAGE pathway and likely do not confer increased disease severity via this pathway.

IL-17 signalling, encompassing all isoforms, is a pro-inflammatory response attracting chemokines and activating cascades to recruit immune cells to sites of inflammation [122]. Exposure to SARS-CoV-2 increased many genes within the IL-17 signalling pathway [32, 66, 91]. Increases in IL-17 are observed in COVID-19 patients and associated with the cytokine storm and ARDS, with IL-17 blockers being investigated as potential treatments in patients with severe COVID-19 [123]. Similarly, exposure to CS or nicotine upregulated most of the IL-17 signalling pathway, including CXCL8, MAPK1 and MUC5AC [18, 22, 53, 67, 69-71, 74, 78-83, 85, 90, 100, 124-137]. Elevation of Il-17 signalling with further exacerbation following SARS-CoV-2 infection, could suggest that smokers may experience more severe disease. Whereas, the limited data available suggested EC+N and EC-N, do not upregulate IL-17 signalling and so are also unlikely to confer increased disease severity via this pathway.

It is worth noting that there was overlap in the GOI within, and thus potential cross-talk between, the VEGF, AGE-RAGE and IL-17 pathways. This highlights both the complexity of cellular responses to CS,

e-cigarettes or nicotine and the need to further investigate and validate specific mechanisms in human relevant-models with appropriate controls and gene/pathway activators/inhibitors.

4.4 Impact of smoking/vaping on the risk/severity of SARS-CoV-2 infection

Overall, the mechanistic evidence to-date suggests that cigarette smokers may be at a higher risk of both infection and more severe disease, supporting recently published literature reviews assessing patient outcomes and the potential impact of smoking on such outcomes [138, 139]. While the data on e-cigarettes is limited, there is evidence for a potential increased risk of infection and/or disease severity in vapers of EC+N, with vapers possibly at a lower risk of developing severe COVID-19 *vs* smokers but at increased risk *vs* non-smokers. This highlights a key role for nicotine-mediated mechanisms in the health impacts of smoking and vaping.

4.5 Other infections / diseases

Pathway analysis also identified other disease, and infection, related pathways, including human cytomegalovirus, influenza A, Kaposi sarcoma-associated herpesvirus infection and Chagas disease. Both human cytomegalovirus and influenza A are more prevalent in smokers [140-143], providing further support that the results of this review are applicable to wider respiratory infections. In contrast, CS appears to have an inverse relationship with Kaposi sarcoma-associated herpesvirus infection and cancer development [144-146] likely due to virus- and/or disease-specific mechanisms. Chagas disease is a parasitic vector borne disease that causes immunoinflammatory-driven fibrosis, particularly in the myocardium and digestive system [147], where smoking has been speculated as an underlying risk factor for aspects of severe disease [148]. This highlights the robustness of this review and the methods used, further demonstrates the complexity and variety of downstream responses to smoking and highlights the importance of investigating smoking and vaping related impacts on other communicable diseases.

4.6 Recommendations for future work

The key challenges and knowledge gaps highlighted by this review include study design, lack of studies on e-cigarettes, building on existing literature, (including additional cigarette constituents, cell types and/or genes and pathways of interest), risk of infection *vs* disease severity, and application to other infections/diseases. Therefore, we recommend that future work should consider:

1. Study design: Should address the specific research question within the most physiologically- and exposure-relevant model where possible. Models should be 1) selected according to airway region, cell types, sex differences, normal *vs* disease and expression of genes or pathways of interest, and 2) fully characterised and validated. Resulting publications should clearly state the justification for the specific model, exposure route, dose(s) administered, and endpoints profiled to aid comparison across different studies. It is also important to include studies on normal/healthy models since these are essential to understanding mechanisms before targeting specific populations.

2. E-cigarettes: More research on the cellular responses to e-cigarettes is needed, particularly on genes and pathways of interest highlighted in this review where data was unavailable (AKT1, CDH1, CHRNA5, CXCL8, IL6, NFKB1 and TNF). These studies must compare EC+N and EC-N to further elucidate the role of nicotine in the health impacts of smoking *vs* vaping.

3. Building on existing literature: Develop a list of 'core genes/pathways' to further investigate with specific hypothesis driven studies. This would add to the weight of existing evidence, enable better comparison between studies and could evolve with the expanding literature. The safety of new products, such as e-cigarettes with different compositions or flavours, could then be more rapidly compared to existing products with known impacts. This is particularly pertinent following recent evidence that other constituents of e-cigarette aerosols can impact susceptibility to SARS-CoV-2 infection[149]. The literature should therefore be continually reviewed to identify additional cigarette ingredients/compositions (eg benzoic acid), cell types (eg endothelial and immune cells), genes and/or pathways (eg oxidative stress and antioxidant mechanisms) of interest as the evidence grows.

4. Risk of infection *vs* disease severity: Better understand how the processes of infection and subsequent disease development inter-relate and are impacted by smoking/vaping and wider environmental exposures.

5. Other infections/diseases: Similar reviews, incorporating a weight-of-evidence based approach that considers the frequency, consistency and quality of existing literature, should be performed to assess the impact of smoking and vaping on wider infections and diseases with inflammatory mechanisms.

5.0 Conclusions

To our knowledge, this is the first review to assess mechanistic associations between smoking or vaping and SARS-CoV-2 infection and disease severity. Using a novel weight-of-evidence meta-analysis, we have identified genes and pathways of interest within the respiratory tract altered by smoking, vaping and/or nicotine that may impact SARS-CoV-2 infection and/or resulting COVID-19 severity. This suggests that cigarette smokers may be at a higher risk of both infection and more severe disease. Large knowledge gaps remain on the impact of e-cigarettes, with the limited data suggesting a potential increased risk of infection and/or disease severity in vapers of e-cigarettes, particularly those containing nicotine. This highlights a key role for nicotine-mediated mechanisms in the health impacts of smoking and vaping. Further specific hypothesis-driven experimental investigations within more physiologically-relevant models and improved study design reporting are required to build on our existing knowledge and promote comparisons across studies. Such work is essential for developing improved public health guidance on the risk of communicable disease infection and severity for potentially more vulnerable populations such as smokers and vapers.

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Conflicts of interest

No conflicts of interest to disclose.

Supplementary information

Supplementary information 1 - Search string

Supplementary information 2 - Data extraction summary

Supplementary information 3 - Quality scoring tool

Supplementary information 4 - Quality assessment summary

Supplementary information 5 - Confidence scoring of genes

Table

Table 1: Inclusion and exclusion criteria for selected studies.

Included studies:	Excluded studies:
• Primary literature articles published in English • Focused on epithelial cells within the respiratory tract and exposure to cigarette smoke, e-cigarettes, nicotine and/or a respiratory virus	• Focused on either epithelial mesenchymal transition, chronic obstructive pulmonary disease (COPD), cancer, pregnancy, cystic fibrosis, pulmonary sarcoidosis, or other respiratory cell types such as endothelial cells • Used cancer-derived cell lines or samples from patients with comorbidities such as lung cancer or COPD • Did not include exposure information • Studied bronchial alveolar lavage or immune cells only

Glossary:

CS	Tobacco cigarette smoke or tobacco cigarette smoke condensate
E-cigarette	Vaping, e-liquid, e-liquid condensate or e-cigarette vape including those with and without nicotine
EC-N	Vaping, e-liquid, e-liquid condensate or e-cigarette vape without nicotine
EC+N	Vaping, e-liquid, e-liquid condensate or e-cigarette vape containing nicotine

REFERENCES

1. WHO. Coronavirus disease (COVID-19) Fact sheet. 2023 09/08/2023.
2. Ni W, Yang X, Yang D, Bao J, Li R, Xiao Y, et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Critical Care. 2020;24(1):1-10.
3. Haslbauer JD, Stalder A, Zinner C, Bassetti S, Mertz KD, Went P, et al. Immunohistochemical and Transcriptional Analysis of SARS-CoV-2 Entry Factors and Renin-Angiotensin-Aldosterone System Components in Lethal COVID-19. Pathobiology. 2022;89(3):166-77.
4. Bayati A, Kumar R, Francis V, McPherson PS. SARS-CoV-2 infects cells after viral entry via clathrin-mediated endocytosis. Journal of Biological Chemistry. 2021;296.
5. Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. Molecular cell. 2020;78(4):779-84. e5.
6. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. cell. 2020;181(2):271-80. e8.
7. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nature reviews Molecular cell biology. 2022;23(1):3-20.
8. Li Z-l, Buck M. Neuropilin-1 assists SARS-CoV-2 infection by stimulating the separation of Spike protein S1 and S2. Biophysical Journal. 2021;120(14):2828-37.
9. Sungnak W, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nature medicine. 2020;26(5):681-7.
10. Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science. 2020;370(6518):856-60.
11. Acosta MAT, Singer BD. Pathogenesis of COVID-19-induced ARDS: implications for an ageing population. European Respiratory Journal. 2020;56(3).
12. Neira DP, Watts A, Seashore J, Polychronopoulou E, Kuo Y-F, Sharma G. Smoking and risk of COVID-19 hospitalization. Respiratory medicine. 2021;182:106414.
13. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, et al. Clinical characteristics of coronavirus disease 2019 in China. New England journal of medicine. 2020;382(18):1708-20.
14. Alqahtani JS, Oyelade T, Aldhahir AM, Alghamdi SM, Almehmadi M, Alqahtani AS, et al. Prevalence, severity and mortality associated with COPD and smoking in patients with COVID-19: a rapid systematic review and meta-analysis. PloS one. 2020;15(5):e0233147.
15. Salah HM, Sharma T, Mehta J. Smoking doubles the mortality risk in COVID-19: a meta-analysis of recent reports and potential mechanisms. Cureus. 2020;12(10).
16. Oakes JM, Fuchs RM, Gardner JD, Lazartigues E, Yue X. Nicotine and the renin-angiotensin system. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2018;315(5):R895-R906.
17. Yue X, Basting TM, Flanagan TW, Xu J, Lobell TD, Gilpin NW, et al. Nicotine downregulates the compensatory angiotensin-converting enzyme 2/angiotensin type 2 receptor of the renin–angiotensin system. Annals of the American Thoracic Society. 2018;15(Supplement 2):S126-S7.
18. Hung Y-H, Hsieh W-Y, Hsieh J-S, Liu F-C, Tsai C-H, Lu L-C, et al. Alternative Roles of STAT3 and MAPK Signaling Pathways in the MMPs Activation and Progression of Lung Injury Induced by Cigarette Smoke Exposure in ACE2 Knockout Mice. International Journal of Biological Sciences. 2016;12(4):454-65.
19. Muus C, Luecken MD, Eraslan G, Waghray A, Heimberg G, Sikkema L, et al. Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells. BioRxiv. 2020:2020.04. 19.049254.
20. Patra T, Meyer K, Geerling L, Isbell TS, Hoft DF, Brien J, et al. SARS-CoV-2 spike protein promotes IL-6 trans-signaling by activation of angiotensin II receptor signaling in epithelial cells. PLoS pathogens. 2020;16(12):e1009128.

21. Arnott D. Use of e-cigarettes (vapes) among young people in Great Britain-June 2023. 2023.
22. Masso-Silva JA, Moshensky A, Shin J, Olay J, Nilaad S, Advani I, et al. Chronic E-Cigarette Aerosol Inhalation Alters the Immune State of the Lungs and Increases ACE2 Expression, Raising Concern for Altered Response and Susceptibility to SARS-CoV-2. Frontiers in Physiology. 2021;12.
23. McAlinden KD, Lu W, Ferdowsi PV, Myers S, Markos J, Larby J, et al. Electronic Cigarette Aerosol Is Cytotoxic and Increases ACE2 Expression on Human Airway Epithelial Cells: Implications for SARS-CoV-2 (COVID-19). Journal of Clinical Medicine. 2021;10(5).
24. Anees-Hill S, Douglas P, Pashley CH, Hansell A, Marczylo EL. A systematic review of outdoor airborne fungal spore seasonality across Europe and the implications for health. Science of the Total Environment. 2022;818:151716.
25. Goode EJ, Marczylo E. A scoping review: What are the cellular mechanisms that drive the allergic inflammatory response to fungal allergens in the lung epithelium? Clinical and Translational Allergy. 2023;13(6):e12252.
26. Baldassi D, Gabold B, Merkel OM. Air− liquid Interface cultures of the healthy and diseased human respiratory tract: promises, challenges, and future directions. Advanced nanobiomed research. 2021;1(6):2000111.
27. Leung C, Wadsworth SJ, Yang SJ, Dorscheid DR. Structural and functional variations in human bronchial epithelial cells cultured in air-liquid interface using different growth media. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2020;318(5):L1063-L73.
28. Wick KD, Fang X, Maishan M, Matsumoto S, Spottiswoode N, Sarma A, et al. Impact of e-cigarette aerosol on primary human alveolar epithelial type 2 cells. American journal of physiology Lung cellular and molecular physiology. 2022;323(2):L152-L64.
29. Palacios-Rápalo SN, De Jesus-Gonzalez LA, Cordero-Rivera CD, Farfan-Morales CN, Osuna-Ramos JF, Martinez-Mier G, et al. Cholesterol-rich lipid rafts as platforms for SARS-CoV-2 entry. Frontiers in immunology. 2021;12:796855.
30. Ghosh A, Girish V, Yuan ML, Coakley RD, Wrennall JA, Alexis NE, et al. Combustible and electronic cigarette exposures increase ACE2 activity and SARS-CoV-2 Spike binding. American Journal of Respiratory and Critical Care Medicine. 2022;205(1):129-33.
31. Li D, Xiong L, Pan G, Wang T, Li R, Zhu L, et al. Molecular imaging on ACE2-dependent transocular infection of coronavirus. Journal of Medical Virology. 2022;94(10):4878-89.
32. Li G, He X, Zhang L, Ran Q, Wang J, Xiong A, et al. Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19. Journal of Autoimmunity. 2020;112.
33. Lv J, Yu P, Wang Z, Deng W, Bao L, Liu J, et al. ACE2 expression is regulated by AhR in SARS-CoV-2-infected macaques. Cellular and Molecular Immunology. 2021;18(5):1308-10.
34. Yang J, Perez EA, Hou C, Zhang P, Van Scoyk M, Winn RA, et al. Identification of the SARS-CoV-2 Entry Receptor ACE2 as a Direct Target for Transcriptional Repression by Miz1. Frontiers in Immunology. 2021;12.
35. Wang G, Zhao Q, Zhang H, Liang F, Zhang C, Wang J, et al. Degradation of SARS-CoV-2 receptor ACE2 by the E3 ubiquitin ligase Skp2 in lung epithelial cells. Frontiers of Medicine. 2021;15(2):252-63.
36. Liu A, Zhang X, Li R, Zheng M, Yang S, Dai L, et al. Overexpression of the SARS-CoV-2 receptor ACE2 is induced by cigarette smoke in bronchial and alveolar epithelia. Journal of Pathology. 2021;253(1):17-30.
37. Lin C-R, Bahmed K, Simborio H, Hayek H, Bolla S, Marchetti N, et al. Expression of SARS-CoV-2 Entry Factors in Human Alveolar Type II Cells in Aging and Emphysema. Biomedicines. 2021;9(7).
38. Leung JM, Yang CX, Sin DD. COVID-19 and nicotine as a mediator of ACE-2. European Respiratory Journal. 2020;55(6).
39. Lee AC, Chakladar J, Li WT, Chen C, Chang EY, Wang-Rodriguez J, et al. Tobacco, but Not Nicotine and Flavor-Less Electronic Cigarettes, Induces ACE2 and Immune Dysregulation. International Journal of Molecular Sciences. 2020;21(15).

40. Chakladar J, Shende N, Li WT, Rajasekaran M, Chang EY, Ongkeko WM. Smoking-Mediated Upregulation of the Androgen Pathway Leads to Increased SARS-CoV-2 Susceptibility. International Journal of Molecular Sciences. 2020;21(10).
41. Cai G, Bosse Y, Xiao F, Kheradmand F, Amos CI. Tobacco Smoking Increases the Lung Gene Expression of ACE2, the Receptor of SARS-CoV-2. American Journal of Respiratory and Critical Care Medicine. 2020;201(12):1557-9.
42. Aliee H, Massip F, Qi C, Stella de Biase M, van Nijnatten JL, Kersten ETG, et al. Determinants of SARS-CoV-2 receptor gene expression in upper and lower airways. medRxiv : the preprint server for health sciences. 2020.
43. Peng Y, Zhao-Ni W, Chen SY, Xu AR, Fang ZF, Sun J, et al. Angiotensin-converting enzyme 2 in peripheral lung club cells modulates the susceptibility to SARS-CoV-2 in chronic obstructive pulmonary disease. American Journal of Physiology - Lung Cellular and Molecular Physiology. 2022;322(5):L712-L21.
44. Qiao Y, Wang X-M, Mannan R, Pitchiaya S, Zhang Y, Wotring JW, et al. Targeting transcriptional regulation of SARS-CoV-2 entry factors ACE2 and TMPRSS2. Proceedings of the National Academy of Sciences of the United States of America. 2021;118(1).
45. Li W, Zheng R, Liang R, Wu B, Wang C, Zhuo L, et al. Effects of Smoking on ACE2 Expression Pattern: Risk and Severity of SARS-CoV-2 Infection. Clinical Laboratory. 2021;67(7):1633-40.
46. Al-Wadei HAN, Al-Wadei MH, Masi T, Schuller HM. Chronic exposure to estrogen and the tobacco carcinogen NNK cooperatively modulates nicotinic receptors in small airway epithelial cells. Lung Cancer. 2010;69(1):33-9.
47. Nakayama T, Lee IT, Jiang S, Matter MS, Yan CH, Overdevest JB, et al. Determinants of SARS-CoV-2 entry and replication in airway mucosal tissue and susceptibility in smokers. Cell Reports Medicine. 2021;2(10).
48. Lallai V, Manca L, Fowler CD. E-cigarette vape and lung ACE2 expression: Implications for coronavirus vulnerability. Environmental Toxicology and Pharmacology. 2021;86.
49. Wang Q, Sundar IK, Li D, Lucas JH, Muthumalage T, McDonough SR, et al. E-cigarette-induced pulmonary inflammation and dysregulated repair are mediated by nAChR alpha 7 receptor: role of nAChR alpha 7 in SARS-CoV-2 Covid-19 ACE2 receptor regulation. Respiratory Research. 2020;21(1).
50. Phandthong R, Wong M, Song A, Martinez T, Talbot P. New Insights into How JUUL Electronic Cigarette Aerosols and Aerosol Constituents Affect SARS-CoV-2 Infection of Human Bronchial Epithelial Cells. bioRxiv : the preprint server for biology. 2022.
51. West KA, Brognard J, Clark AS, Linnoila IR, Yang XW, Swain SM, et al. Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. Journal of Clinical Investigation. 2003;111(1):81-90.
52. Lupacchini L, Maggi F, Tomino C, De Dominicis C, Mollinari C, Fini M, et al. Nicotine Changes Airway Epithelial Phenotype and May Increase the SARS-COV-2 Infection Severity. Molecules. 2020;26(1).
53. Carlisle DL, Hopkins TM, Gaither-Davis A, Silhanek MJ, Luketich JD, Christie NA, et al. Nicotine signals through muscle-type and neuronal nicotinic acetylcholine receptors in both human bronchial epithelial cells and airway fibroblasts. Respiratory Research. 2004;5(27-28).
54. Zia S, Ndoye A, Nguyen VT, Grando SA. Nicotine enhances expression of the alpha 3, alpha 4, alpha 5, and alpha 7 nicotinic receptors modulating calcium metabolism and regulating adhesion and motility of respiratory epithelial cells. Research Communications in Molecular Pathology and Pharmacology. 1997;97(3):243-62.
55. Russo P, Bonassi S, Giacconi R, Malavolta M, Tomino C, Maggi F. COVID-19 and smoking: Is nicotine the hidden link? European Respiratory Journal. 2020;55(6) (no pagination).
56. Bernardi S, Toffoli B, Zennaro C, Tikellis C, Monticone S, Losurdo P, et al. High-salt diet increases glomerular ACE/ACE2 ratio leading to oxidative stress and kidney damage. Nephrology Dialysis Transplantation. 2012;27(5):1793-800.

57. de Kloet AD, Cahill KM, Scott KA, Krause EG. Overexpression of angiotensin converting enzyme 2 reduces anxiety-like behavior in female mice. Physiology & behavior. 2020;224:113002.
58. Fattahi S, Khalifehzadeh-Esfahani Z, Mohammad-Rezaei M, Mafi S, Jafarinia M. PI3K/Akt/mTOR pathway: a potential target for anti-SARS-CoV-2 therapy. Immunologic Research. 2022;70(3):269-75.
59. Murray JL, McDonald NJ, Sheng J, Shaw MW, Hodge TW, Rubin DH, et al. Inhibition of influenza A virus replication by antagonism of a PI3K-AKT-mTOR pathway member identified by gene-trap insertional mutagenesis. Antiviral Chemistry and Chemotherapy. 2012;22(5):205-15.
60. Kindrachuk J, Ork B, Mazur S, Holbrook MR, Frieman MB, Traynor D, et al. Antiviral potential of ERK/MAPK and PI3K/AKT/mTOR signaling modulation for Middle East respiratory syndrome coronavirus infection as identified by temporal kinome analysis. Antimicrobial agents and chemotherapy. 2015;59(2):1088-99.
61. Abdelrahman Z, Li M, Wang X. Comparative review of SARS-CoV-2, SARS-CoV, MERS-CoV, and influenza a respiratory viruses. Frontiers in immunology. 2020:2309.
62. Shen H-j, Jiang J-x, Yao H-y, Zhou H-y, Xie Q-m, Dai H-b, et al. Glycopyrronium bromide regulates cigarette smoke-induced epithelial mesenchymal transition by mediating ACh production. Journal of Molecular Structure. 2022;1266.
63. Wang Q, Wang H, Zhang Y, Xiao W. Activation of uPAR is required for cigarette smoke extract-induced epithelial-mesenchymal transition in lung epithelial cells. Oncology Research. 2014;21(6):295-305.
64. Li E, Xu Z, Liu F, Wang H, Wen J, Shao S, et al. Continual Exposure to Cigarette Smoke Extracts Induces Tumor-Like Transformation of Human Nontumor Bronchial Epithelial Cells in a Microfluidic Chip. Journal of Thoracic Oncology. 2014;9(8):1091-100.
65. Guo JJ, Chu M, Abbeyquaye T, Chen CY. Persistent nicotine treatment potentiates amplification of the dihydrofolate reductase gene in rat lung epithelial cells as a consequence of Ras activation. Journal of Biological Chemistry. 2005;280(34):30422-31.
66. Li F, Li J, Wang P-H, Yang N, Huang J, Ou J, et al. SARS-CoV-2 spike promotes inflammation and apoptosis through autophagy by ROS-suppressed PI3K/AKT/mTOR signaling. Biochimica Et Biophysica Acta-Molecular Basis of Disease. 2021;1867(12).
67. Li H, Cui L, Liu Q, Dou S, Wang W, Xie M, et al. Ginsenoside Rb3 Alleviates CSE-induced TROP2 Upregulation through p38 MAPK and NF-kappa B Pathways in Basal Cells. American Journal of Respiratory Cell and Molecular Biology. 2021;64(6):747-59.
68. Chen P, Jiang P, Chen J, Yang Y, Guo X. XIST promotes apoptosis and the inflammatory response in CSE-stimulated cells via the miR-200c-3p/EGR3 axis. Bmc Pulmonary Medicine. 2021;21(1).
69. Hou W, Hu S, Yong KT, Zhang J, Ma H. Cigarette smoke-induced malignant transformation via STAT3 signalling in pulmonary epithelial cells in a lung-on-a-chip model. Bio-Design and Manufacturing. 2020;3(4):383-95.
70. Van der Toorn M, Marescotti D, Johne S, Baumer K, Bornand D, Dulize R, et al. Comparison of the Biological Effects of Long-Term Exposure of Human Bronchial Epithelial Cells to Total Particulate Matter From 3R4F Cigarette Smoke or Aerosol From the Candidate Modified Risk Tobacco Product (cMRTP) THS2.2. International Journal of Toxicology. 2017;36(1):66-7.
71. Jiang J-X, Zhang S-J, Shen H-J, Guan Y, Liu Q, Zhao W, et al. Racl signaling regulates cigarette smoke-induced inflammation in the lung via the Erk1/2 MAPK and STAT3 pathways. Biochimica Et Biophysica Acta-Molecular Basis of Disease. 2017;1863(7):1778-88.
72. Mishra R, Foster D, Vasu VT, Thaikoottathil JV, Kosmider B, Chu HW, et al. Cigarette Smoke Induces Human Epidermal Receptor 2-Dependent Changes in Epithelial Permeability. American Journal of Respiratory Cell and Molecular Biology. 2016;54(6):853-64.
73. Foronjy RF, Salathe MA, Dabo AJ, Baumlin N, Cummins N, Eden E, et al. TLR9 expression is required for the development of cigarette smoke-induced emphysema in mice. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2016;311(1):L154-L66.

74. Geraghty P, Hardigan A, Foronjy RF. Cigarette Smoke Activates the Proto-oncogene c-Src to Promote Airway Inflammation and Lung Tissue Destruction. American Journal of Respiratory Cell and Molecular Biology. 2014;50(3):559-70.
75. Tang X, Li Z, Yu Z, Li J, Zhang J, Wan N, et al. Effect of curcumin on lung epithelial injury and ferroptosis induced by cigarette smoke. Human & Experimental Toxicology. 2021;40(12_SUPPL):S753-S62.
76. Zhao Y, Xu Y, Li Y, Xu W, Luo F, Wang B, et al. NF-kappaB-mediated inflammation leading to EMT via miR-200c is involved in cell transformation induced by cigarette smoke extract. Toxicological Sciences. 2013;135(2):265-76.
77. Tsutsumi A, Ozaki M, Chubachi S, Irie H, Sato M, Kameyama N, et al. Exposure to Cigarette Smoke Enhances the Sternness of Alveolar Type 2 Cells. American Journal of Respiratory Cell and Molecular Biology. 2020;63(3):293-305.
78. Vivarelli F, Canistro D, Cirillo S, Elias RJ, Granata S, Mussoni M, et al. Unburned Tobacco Cigarette Smoke Alters Rat Ultrastructural Lung Airways and DNA. Nicotine & Tobacco Research. 2021;23(12):2127-34.
79. Wohnhaas CT, Gindele JA, Kiechle T, Shen Y, Leparc GG, Stierstorfer B, et al. Cigarette Smoke Specifically Affects Small Airway Epithelial Cell Populations and Triggers the Expansion of Inflammatory and Squamous Differentiation Associated Basal Cells. International Journal of Molecular Sciences. 2021;22(14).
80. Albano GD, Bonanno A, Moscato M, Anzalone G, Di Sano C, Riccobono L, et al. Crosstalk between mAChRM3 and beta 2AR, via acetylcholine PI3/PKC/PBEP1/Raf-1 MEK1/2/ERK1/2 pathway activation, in human bronchial epithelial cells after long-term cigarette smoke exposure. Life Sciences. 2018;192:99-109.
81. Li F-f, Shen J, Shen H-j, Zhang X, Cao R, Zhang Y, et al. Shp2 Plays an Important Role in Acute Cigarette Smoke-Mediated Lung Inflammation. Journal of Immunology. 2012;189(6):3159-67.
82. Carlier FM, Detry B, Lecocq M, Collin AM, Plante-Bordeneuve T, Verleden SE, et al. The memory of airway epithelium damage in smokers and COPD patients. bioRxiv. 2021;22.
83. Ho YS, Chen CH, Wang YJ, Pestell RG, Albanese C, Chen RJ, et al. Tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces cell proliferation in normal human bronchial epithelial cells through NF kappa B activation and cyclin D1 up-regulation. Toxicology and Applied Pharmacology. 2005;205(2):133-48.
84. Ma L, Jiang M, Zhao X, Sun J, Pan Q, Chu S. Cigarette and IL-17A synergistically induce bronchial epithelial-mesenchymal transition via activating IL-17R/NF-kappa B signaling. Bmc Pulmonary Medicine. 2020;20(1).
85. Sarkar P, Hayes BE. Induction of COX-2 by acrolein in rat lung epithelial cells. Molecular and Cellular Biochemistry. 2007;301(1-2):191-9.
86. Arredondo J, Chernyavsky AI, Grando SA. The nicotinic receptor antagonists abolish pathobiologic effects of tobacco-derived nitrosamines on BEP2D cells. Journal of Cancer Research and Clinical Oncology. 2006;132(10):653-63.
87. Hirschi-Budge KM, Tsai KYF, Curtis KL, Davis GS, Theurer BK, Kruyer AMM, et al. RAGE signaling during tobacco smoke-induced lung inflammation and potential therapeutic utility of SAGEs. Bmc Pulmonary Medicine. 2022;22(1).
88. Li Q, Zhou XD, Kolosov VP, Perelman JM. Nicotine reduces TNF-alpha expression through a alpha7 nAChR/MyD88/NF-kappaB pathway in HBE16 airway epithelial cells. Cellular Physiology and Biochemistry. 2011;27(5):605-12.
89. Ebrahimpour A, Shrestha S, Bonnen MD, Eissa NT, Raghu G, Ghebre YT. Nicotine Modulates Growth Factors and MicroRNA to Promote Inflammatory and Fibrotic Processes. Journal of Pharmacology and Experimental Therapeutics. 2019;368(2):169-78.
90. Li Q, Zhou X, Kolosov VP, Perelman JM. The Expression and Pharmacological Characterization of Nicotinic Acetylcholine Receptor Subunits in HBE16 Airway Epithelial Cells. Cell Biochemistry and Biophysics. 2012;62(3):421-31.

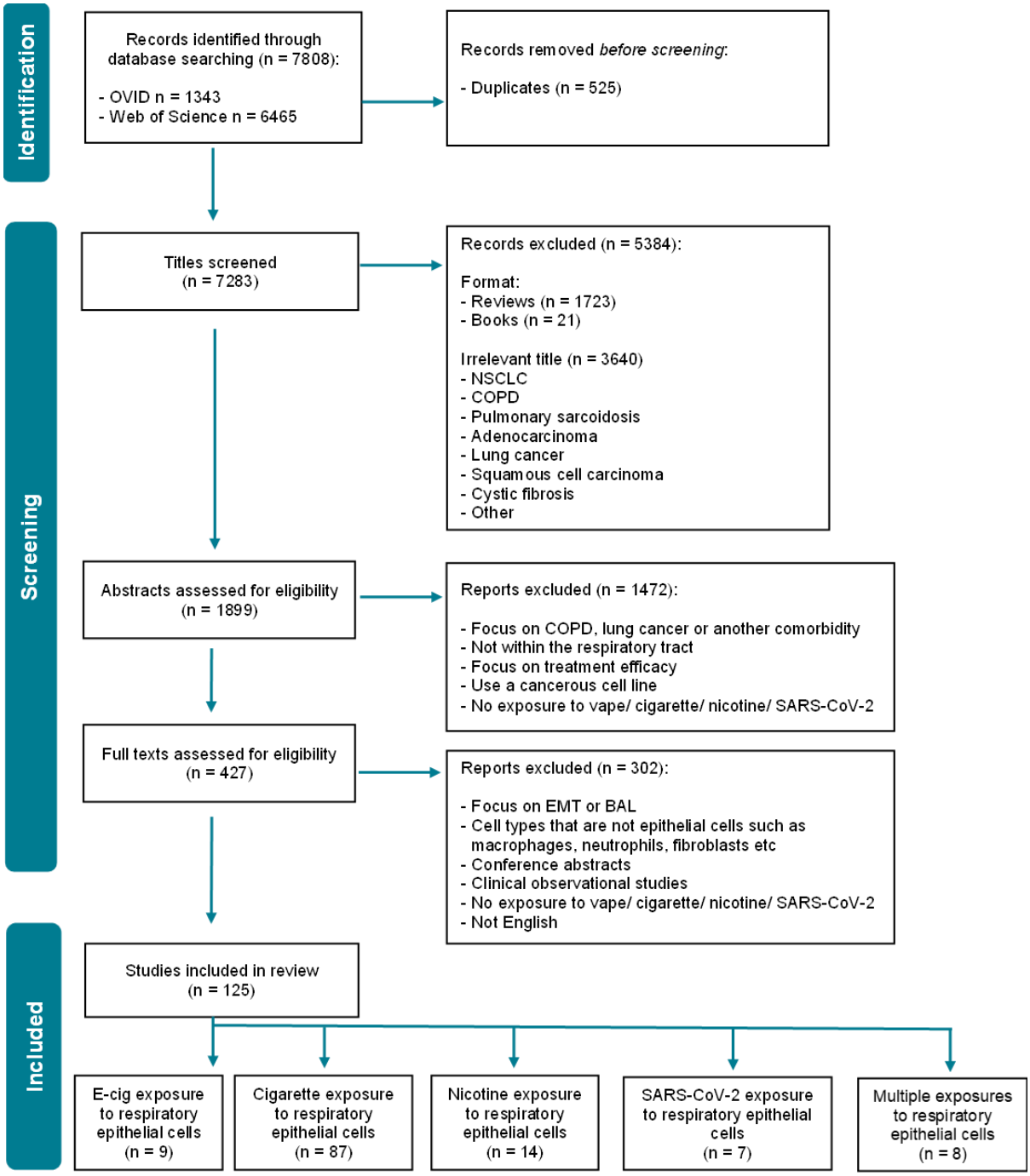
91. Chen Q, Huang X-Y, Tian Y, Fan C, Sun M, Zhou C, et al. The Infection and Pathogenicity of SARS-CoV-2 Variant B.1.351 in hACE2 Mice. Virologica Sinica. 2021;36(5):1232-5.
92. Potere N, Batticciotto A, Vecchié A, Porreca E, Cappelli A, Abbate A, et al. The role of IL-6 and IL-6 blockade in COVID-19. Expert review of clinical immunology. 2021;17(6):601-18.
93. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. The Journal of clinical investigation. 2020;130(5):2620-9.
94. Camblor DG, Miranda D, Albaiceta GM, Amado-Rodríguez L, Cuesta-Llavona E, Vázquez-Coto D, et al. Genetic variants in the NF-κB signaling pathway (NFKB1, NFKBIA, NFKBIZ) and risk of critical outcome among COVID-19 patients. Human immunology. 2022;83(8-9):613-7.
95. Cesta MC, Zippoli M, Marsiglia C, Gavioli EM, Cremonesi G, Khan A, et al. Neutrophil activation and neutrophil extracellular traps (NETs) in COVID-19 ARDS and immunothrombosis. European Journal of Immunology. 2023;53(1):2250010.
96. Wang Y, Wang H, Zhang C, Zhang C, Yang H, Gao R, et al. Lung fluid biomarkers for acute respiratory distress syndrome: a systematic review and meta-analysis. Critical Care. 2019;23(1):1-15.
97. Carolina D, Couto AE, Campos LC, Vasconcelos TF, Michelon-Barbosa J, Corsi CA, et al. MMP-2 and MMP-9 levels in plasma are altered and associated with mortality in COVID-19 patients. Biomedicine & Pharmacotherapy. 2021;142:112067.
98. Tan LH, Bahmed K, Lin C-R, Marchetti N, Bolla S, Criner GJ, et al. The cytoprotective role of DJ-1 and p45 NFE2 against human primary alveolar type II cell injury and emphysema. Scientific Reports. 2018;8.
99. Podowski M, Calvi C, Metzger S, Misono K, Poonyagariyagorn H, Lopez-Mercado A, et al. Angiotensin receptor blockade attenuates cigarette smoke-induced lung injury and rescues lung architecture in mice. Journal of Clinical Investigation. 2012;122(1):229-40.
100. Golovatch P, Mercer BA, Lemaitre V, Wallace A, Foronjy RF, D'Armiento J. ROLE FOR CATHEPSIN K IN EMPHYSEMA IN SMOKE-EXPOSED GUINEA PIGS. Experimental Lung Research. 2009;35(8):631-45.
101. Agraval H, Sharma JR, Prakash N, Yadav UCS. Fisetin suppresses cigarette smoke extract-induced epithelial to mesenchymal transition of airway epithelial cells through regulating COX-2/MMPs/13-catenin pathway. Chemico-Biological Interactions. 2022;351.
102. Li Z-Z, Guo Z-Z, Zhang Z, Cao Q-A, Zhu Y-J, Yao H-L, et al. Nicotine-induced upregulation of VCAM-1, MMP-2, and MMP-9 through the α7-nAChR-JNK pathway in RAW264. 7 and MOVAS cells. Molecular and cellular biochemistry. 2015;399:49-58.
103. Pagliaro P, Penna C. ACE/ACE2 ratio: a key also in 2019 coronavirus disease (Covid-19)? Frontiers in medicine. 2020;7:335.
104. Alkhouli M, Nanjundappa A, Annie F, Bates MC, Bhatt DL, editors. Sex differences in case fatality rate of COVID-19: insights from a multinational registry. Mayo clinic proceedings; 2020: Elsevier.
105. Sharma G, Volgman AS, Michos ED. Sex differences in mortality from COVID-19 pandemic: are men vulnerable and women protected? Case Reports. 2020;2(9):1407-10.
106. Vadakedath S, Kandi V, Mohapatra RK, Pinnelli VB, Yegurla RR, Shahapur PR, et al. Immunological aspects and gender bias during respiratory viral infections including novel Coronavirus disease-19 (COVID-19): A scoping review. Journal of Medical Virology. 2021;93(9):5295-309.
107. Shibuya M. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti-and pro-angiogenic therapies. Genes & cancer. 2011;2(12):1097-105.
108. Holmes DI, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome biology. 2005;6(2):1-10.
109. Moutal A, Martin LF, Boinon L, Gomez K, Ran D, Zhou Y, et al. SARS-CoV-2 spike protein co-opts VEGF-A/neuropilin-1 receptor signaling to induce analgesia. Pain. 2021;162(1):243.

110. Hamilton JL, Nagao M, Levine BR, Chen D, Olsen BR, Im HJ. Targeting VEGF and its receptors for the treatment of osteoarthritis and associated pain. Journal of Bone and Mineral Research. 2016;31(5):911-24.
111. Hulse RP. Role of VEGF-A in chronic pain. Oncotarget. 2017;8(7):10775.
112. Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nature medicine. 2020;26(8):1200-4.
113. Laboratories K. AGE-RAGE signalling pathway in diabetic complications - Homo sapiens (human) 2019 [Available from: https://www.kegg.jp/pathway/hsa04933.
114. Mengstie MA, Chekol Abebe E, Behaile Teklemariam A, Tilahun Mulu A, Agidew MM, Teshome Azezew M, et al. Endogenous advanced glycation end products in the pathogenesis of chronic diabetic complications. Frontiers in Molecular Biosciences. 2022;9:1002710.
115. Chiappalupi S, Salvadori L, Donato R, Riuzzi F, Sorci G. Hyperactivated RAGE in comorbidities as a risk factor for severe COVID-19—The role of RAGE-Ras crosstalk. Biomolecules. 2021;11(6):876.
116. Sellegounder D, Zafari P, Rajabinejad M, Taghadosi M, Kapahi P. Advanced glycation end products (AGEs) and its receptor, RAGE, modulate age-dependent COVID-19 morbidity and mortality. A review and hypothesis. International immunopharmacology. 2021;98:107806.
117. Chu M, Guo JJ, Chen CY. Long-term exposure to nicotine, via Ras pathway, induces cyclin D1 to stimulate G(1) cell cycle transition. Journal of Biological Chemistry. 2005;280(8):6369-79.
118. Marshall K, Liu Z, Olfert IM, Gao W. Chronic electronic cigarette use elicits molecular changes related to pulmonary pathogenesis. Toxicology and Applied Pharmacology. 2020;406.
119. Zhang Y, Huang W, Zheng Z, Wang W, Yuan Y, Hong Q, et al. Cigarette smoke-inactivated SIRT1 promotes autophagy-dependent senescence of alveolar epithelial type 2 cells to induce pulmonary fibrosis. Free Radical Biology and Medicine. 2021;166:116-27.
120. Maehira F, Miyagi I, Asato T, Eguchi Y, Takei H, Nakatsuki K, et al. Alterations of protein kinase C, 8-hydroxydeoxyguanosine, and K-ras oncogene in rat lungs exposed to passive smoking. Clinica Chimica Acta. 1999;289(1-2):133-44.
121. Ogawa F, Walters MS, Shafquat A, O'Beirne SL, Kaner RJ, Mezey JG, et al. Role of KRAS in regulating normal human airway basal cell differentiation. Respiratory Research. 2019;20(1).
122. Laboratories K. IL-17 signalling patwhay - Homo sapiens (human) 2023 [Available from: https://www.kegg.jp/kegg-bin/show_pathway?hsa04657.
123. Pacha O, Sallman MA, Evans SE. COVID-19: a case for inhibiting IL-17? Nature Reviews Immunology. 2020;20(6):345-6.
124. Cortijo J, Mata M, Milara J, Donet E, Gavalda A, Miralpeix M, et al. Aclidinium inhibits cholinergic and tobacco smoke-induced MUC5AC in human airways. European Respiratory Journal. 2011;37(2):244-54.
125. Gundavarapu S, Wilder JA, Mishra NC, Rir-sima-ah J, Langley RJ, Singh SP, et al. Role of nicotinic receptors and acetylcholine in mucous cell metaplasia, hyperplasia, and airway mucus formation in vitro and in vivo. Journal of Allergy and Clinical Immunology. 2012;130(3):770-+.
126. Li Q, Kolosov VP, Perelman JM, Zhou XD. Src-JNK-dependent pathway in cigarette smoke-induced mucous hypersecretion in airway epithelial cells. Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology. 2010;4(4):363-73.
127. Mebratu YA, Schwalm K, Smith KR, Schuyler M, Tesfaigzi Y. Cigarette Smoke Suppresses Bik To Cause Epithelial Cell Hyperplasia and Mucous Cell Metaplasia. American Journal of Respiratory and Critical Care Medicine. 2011;183(11):1531-8.
128. Monzon ME, Forteza RM, Casalino-Matsuda SM. MCP-1/CCR2B-dependent loop upregulates MUC5AC and MUC5B in human airway epithelium. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2011;300(2):L204-L15.
129. O'Donnell RA, Richter A, Ward J, Angco G, Mehta A, Rousseau K, et al. Expression of ErbB receptors and mucins in the airways of long term current smokers. Thorax. 2004;59(12):1032-40.

130. Polosukhin VV, Cates JM, Lawson WE, Milstone AP, Matafonov AG, Massion PP, et al. Hypoxia-inducible factor-1 signalling promotes goblet cell hyperplasia in airway epithelium. Journal of Pathology. 2011;224(2):203-11.
131. Rayner RE, Wellmerling J, Makena P, Zhao J, Prasad GL, Cormet-Boyaka E. Transcriptomic Response of Primary Human Bronchial Cells to Repeated Exposures of Cigarette and ENDS Preparations. Cell Biochemistry and Biophysics. 2022;80(1):217-28.
132. Voinsky I, Gurwitz D. Smoking andCOVID-19: Similar bronchialACE2andTMPRSS2expression and higherTMPRSS4expression in current versus never smokers. Drug Development Research. 2020;81(8):1073-80.
133. Yu H, Li Q, Kolosov VP, Perelman JM, Zhou X. Regulation of Cigarette Smoke-Induced Mucin Expression by Neuregulin1 beta/ErbB3 Signalling in Human Airway Epithelial Cells. Basic & Clinical Pharmacology & Toxicology. 2011;109(1):63-72.
134. Zhang L, Gallup M, Zlock L, Chen YTF, Finkbeiner WE, McNamara NA. Cigarette Smoke Mediates Nuclear to Cytoplasmic Trafficking of Transcriptional Inhibitor Kaiso through MUC1 and P120-Catenin. American Journal of Pathology. 2016;186(12):3146-59.
135. Andrault P-M, Schamberger AC, Chazeirat T, Sizaret D, Renault J, Staab-Weijnitz CA, et al. Cigarette smoke induces overexpression of active human cathepsin S in lungs from current smokers with or without COPD. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2019;317(5):L625-L38.
136. Chung S, Vu S, Filosto S, Goldkorn T. Src Regulates Cigarette Smoke-Induced Ceramide Generation via Neutral Sphingomyelinase 2 in the Airway Epithelium. American Journal of Respiratory Cell and Molecular Biology. 2015;52(6):738-48.
137. Gebel S, Gerstmayer B, Kuhl P, Borlak J, Meurrens K, Mueller T. The kinetics of transcriptomic changes induced by cigarette smoke in rat lungs reveals a specific program of defense, inflammation, and circadian clock gene expression. Toxicological Sciences. 2006;93(2):422-31.
138. Gallus S, Scala M, Possenti I, Jarach CM, Clancy L, Fernandez E, et al. The role of smoking in COVID-19 progression: a comprehensive meta-analysis. European Respiratory Review. 2023;32(167).
139. Sifat AE, Nozohouri S, Villalba H, Vaidya B, Abbruscato TJ. The Role of Smoking and Nicotine in the Transmission and Pathogenesis of COVID-19. Journal of Pharmacology and Experimental Therapeutics. 2020;375(3):498-509.
140. Styles JN, Converse RR, Griffin SM, Wade TJ, Klein E, Nylander-French LA, et al. Human cytomegalovirus infections are associated with elevated biomarkers of vascular injury. Frontiers in Cellular and Infection Microbiology. 2020;10:334.
141. Lawrence H, Hunter A, Murray R, Lim W, McKeever T. Cigarette smoking and the occurrence of influenza–Systematic review. Journal of Infection. 2019;79(5):401-6.
142. Han L, Ran J, Mak Y-W, Suen LK-P, Lee PH, Peiris JSM, et al. Smoking and influenza-associated morbidity and mortality: a systematic review and meta-analysis. Epidemiology. 2019;30(3):405-17.
143. Dickerson F, Katsafanas E, Origoni A, Newman T, Rowe K, Ziemann RS, et al. Cigarette smoking is associated with Herpesviruses in persons with and without serious mental illness. PloS one. 2023;18(1):e0280443.
144. Ford PW, Hamden KE, Whitman AG, Bryan BA, Chintalgattu V, McCubrey JA, et al. Cigarette smoke concentrate inhibits Kaposi's sarcoma-associated herpesvirus infection. Virus research. 2005;114(1-2):172-6.
145. Anderson LA, Lauria C, Romano N, Brown EE, Whitby D, Graubard BI, et al. Risk factors for classical Kaposi sarcoma in a population-based case-control study in Sicily. Cancer Epidemiology Biomarkers & Prevention. 2008;17(12):3435-43.
146. Luu H, Amirian E, Scheurer M. The interaction between smoking status and highly active antiretroviral therapy (HAART) use on the risk of Kaposi's sarcoma (KS) in a cohort of HIV-infected men. British journal of cancer. 2013;108(5):1173-7.

147. Pérez-Molina JA, Molina I. Chagas disease. The Lancet. 2018;391(10115):82-94.
148. Lage TAR, Tupinambás JT, Pádua LBd, Ferreira MdO, Ferreira AC, Teixeira AL, et al. Stroke in Chagas disease: from pathophysiology to clinical practice. Revista da Sociedade Brasileira de Medicina Tropical. 2022;55:e0575-2021.
149. Phandthong R, Wong M, Song A, Martinez T, Talbot P. Does vaping increase the likelihood of SARS-CoV-2 infection? Paradoxically yes and no. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2024;326(2):L175-L89.

A)



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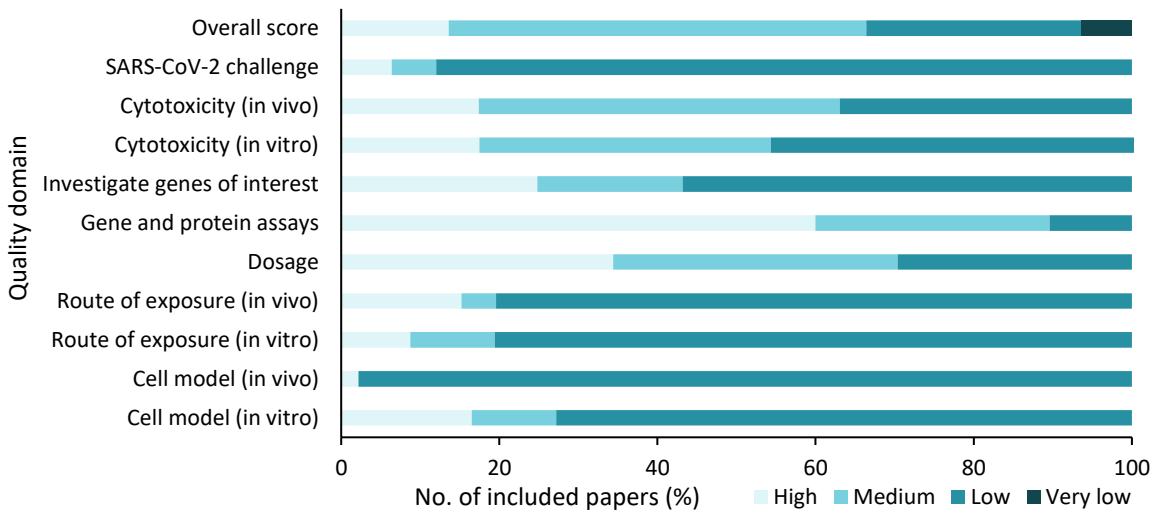


Figure 1: A) PRISMA flowchart displaying the search and selection process for studies included in the systematic review. n = number; e-cig = e-cigarette; COPD = chronic obstructive pulmonary disease; NSCLC = non-small cell lung cancer; EMT = epithelial-mesenchymal transition; BAL = bronchoalveolar lavage. B) A summary of the quality scores assigned to the included studies based on the assessment of domains designed to determine the ability of each study to address the review aims.

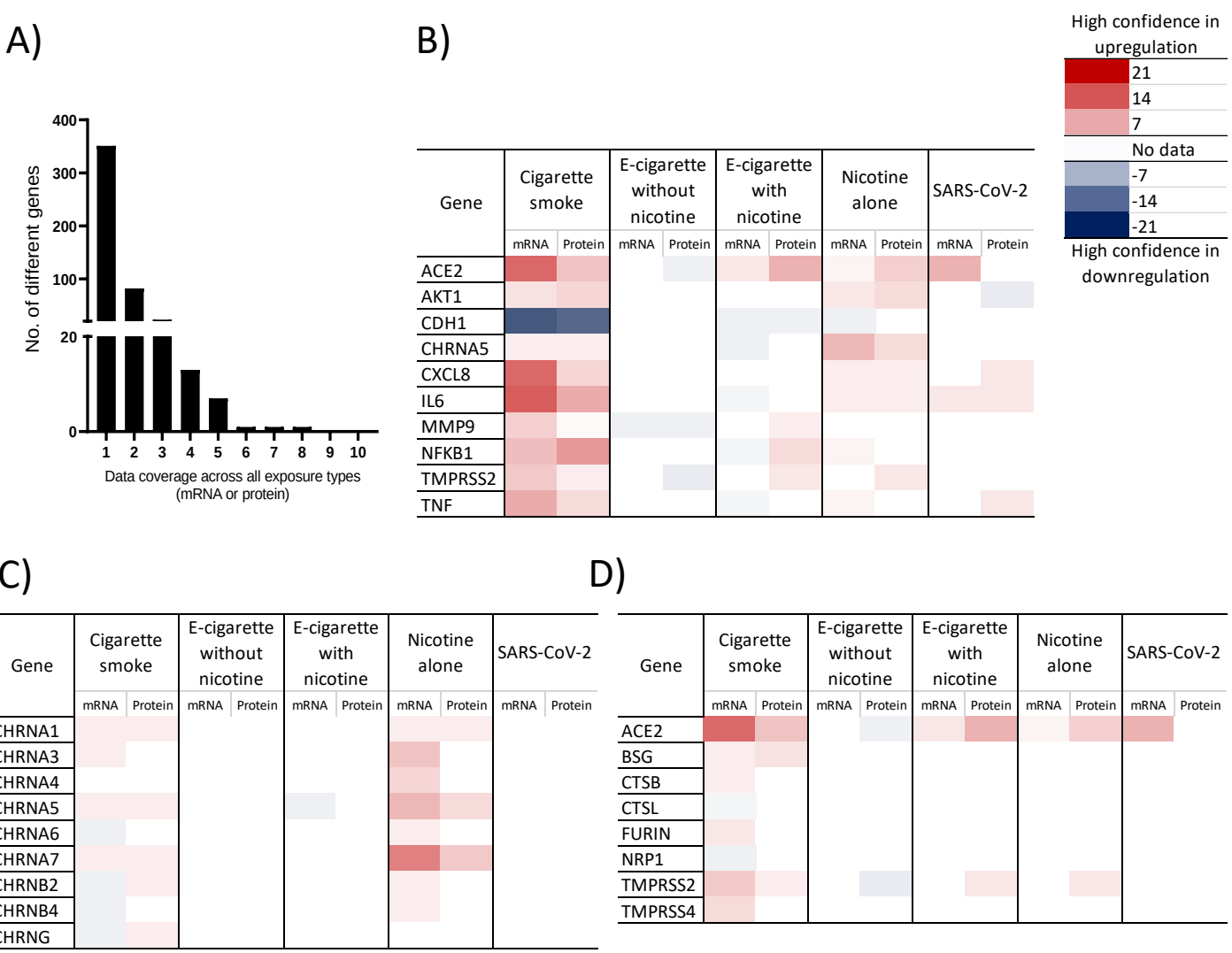
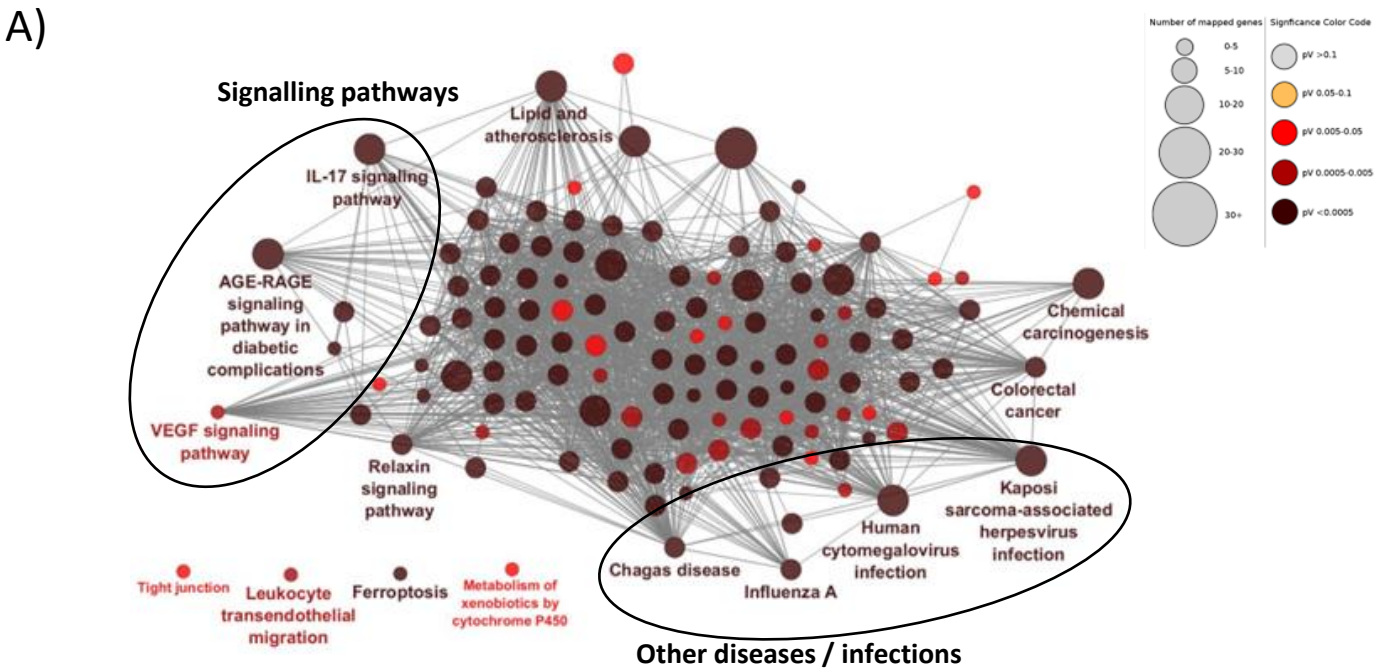


Figure 2: A) The variety of exposure data (mRNA/protein and type) available for each gene. B) The genes with data available for 5 or more fields (mRNA/protein for each exposure type). C) Nicotinic acetylcholine receptor changes following different exposures and D) those known to have roles in SARS-CoV-2 viral entry. GOI = Gene of interest; No. = Number; e-cigarette = Electronic cigarette.



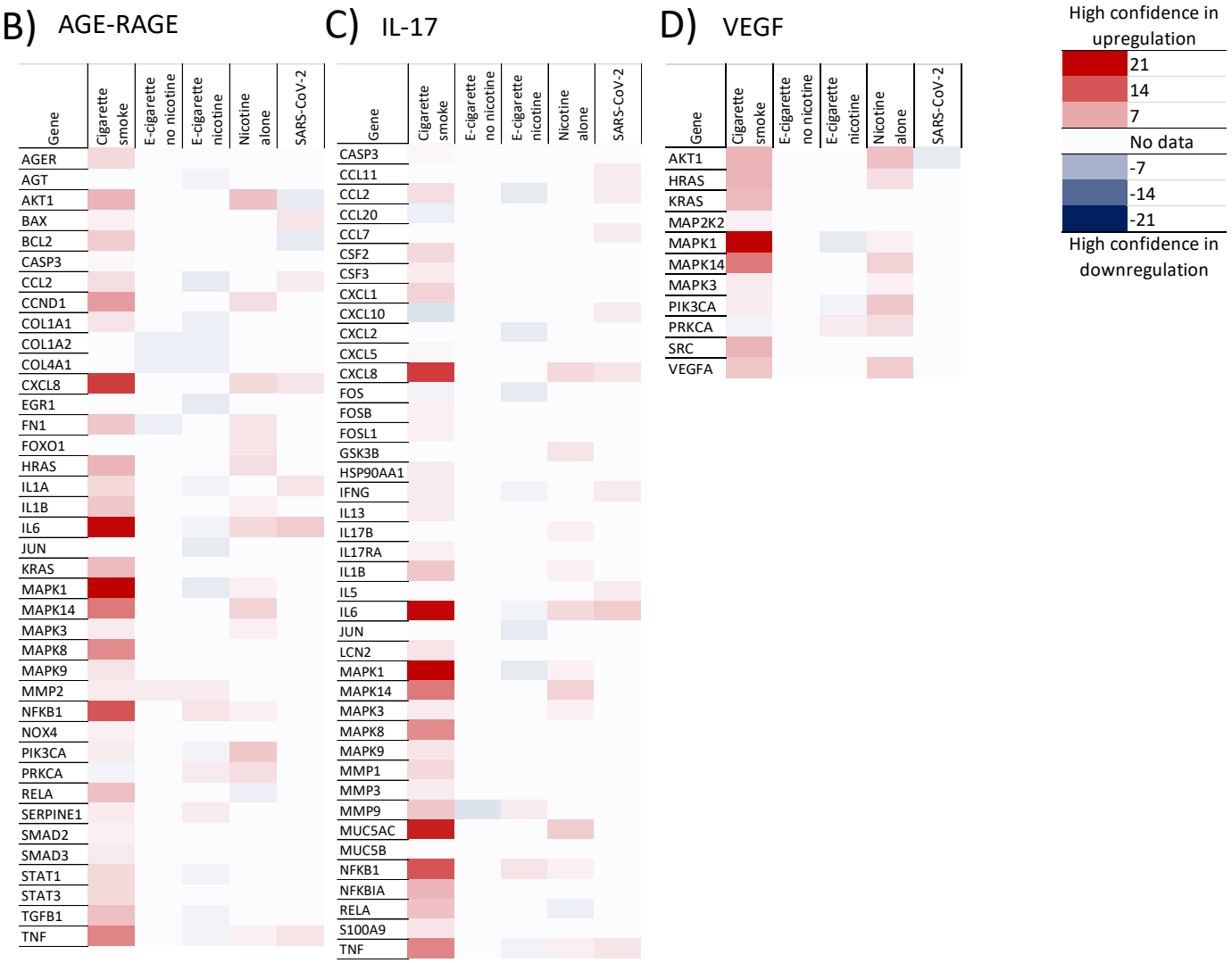
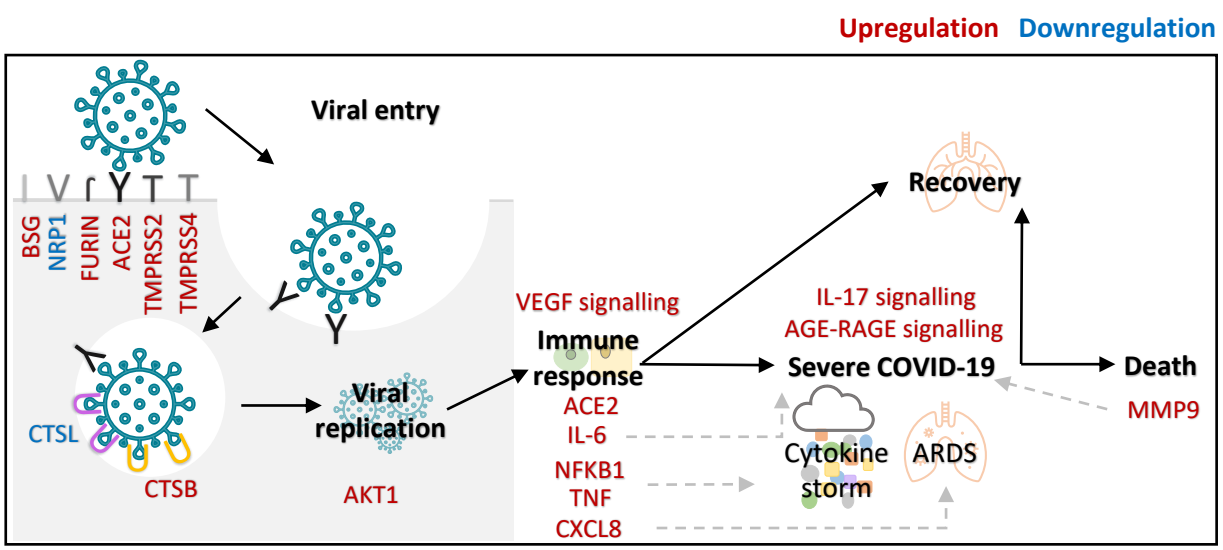


Figure 3: A) Pathway analysis of altered genes following exposure to cigarette smoke, electronic cigarettes and nicotine. B-D) The confidence scores of genes altered following exposure (to cigarette smoke, electronic cigarettes, without or with nicotine and SARS-CoV-2 alone) that are involved in the pathways of interest, AGE-RAGE (in diabetic complications), IL-17 and VEGF signalling.

A) Following exposure to cigarette smoke



B) Following exposure to electronic cigarettes with nicotine

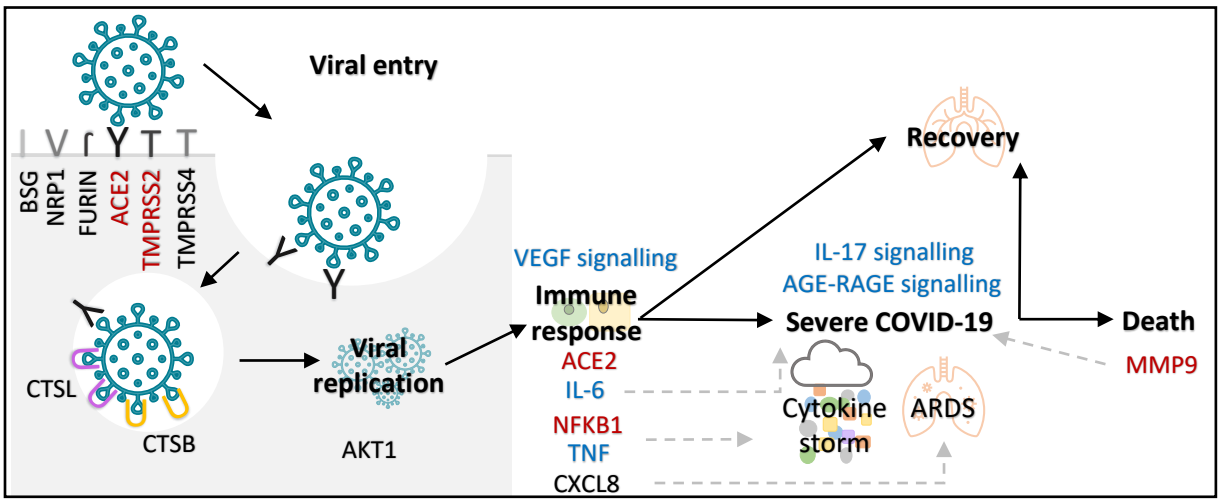


Figure 4 : An overview of how genes and signalling pathways of interest may alter the risk of SARS-CoV-2 infection and subsequent COVID-19 severity following exposure to (A) cigarette smoke or (B) electronic cigarettes with nicotine. Where text remains black for genes or signalling pathways, there was no data available. ARDS = Acute respiratory distress syndrome; COVID-19 = Coronavirus disease 2019.

Supplementary Information for:

Smoking and vaping alter genes related to mechanisms of SARS-CoV-2 susceptibility and severity

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Contents

Supplementary information 1: Search string for the literature review in OVID and Web of Science (WoS) databases. Search A included exposure, pathways and tissue/region of interest; Search B focused on exposure, pathways and respiratory viruses. Results for search A and B within each database were combined up to November 2022. Note due to the large volume of literature results, any key recent studies since November 2022 have been incorporated into the discussion.

Database and search	Joining term	Subject headers **OR** key words (OVID); Key words (WoS)	Total no. results
OVID search A	-	(exp cigarette smoking/ or exp smoking device/ or exp smoking/ or exp cigar smoking/ or exp vaping associated lung injury/ or exp vaping/) **OR** ((smok*/ or vap*/ or cigarette*/ or e-cigarette*/ or electronic-cigarette*/ or e-cig*/ or nicotine end*/ or electronic cigarette*) or (nicotine/ or tobacco/ or ECIG/ or TCIG/ or ENDS) or ("electronic nicotine delivery system") or (cigarette* adj3 smoke*))	1341
	AND	(exp virus entry/ or exp cholinergic system/ or exp renin angiotensin aldosterone system/ or exp "angiotensin[1-7]"/ or exp angiotensin converting enzyme 2/ or exp angiotensin/ or exp epithelial mesenchymal transition) **OR** ((ACE2/ or RAAS/ or ACE/ or TMPRSS2/ or TMPRSS4/ or ADAM17/ or CHRNA7/ or RAS/ or ANG-II/ or ANG-I/ or Furin/ or Cathepsin/ or hACE2/ or MasR/ or NRP1/ or TFRC/ or AXL/ or ASGR1/ or KREMEN1/ or CD147/ or TfR/ or IFN-beta1/ or IFNB1/ or CTSL/ or CTSB/ or AGTR2/ or AT2/ or AGTR1/ or AT1/ or MAS1/ or nAChRs/ or EMT/ or LZTFL1/ or SNAI*/ or ZEB2/ or IFI27) or ("angiotensin converting enzyme 2"/ or "renin angiotensin aldosterone system"/ or "renin-angiotensin-aldosterone system"/ or "angiotensin converting enzyme"/ or "transmembrane serine protease 2"/ or "transmembrane serine protease 4"/ or "ADAM metallopeptidase Domain 17"/ or "Nicotinic acid receptor"/ or "cholinergic receptor nicotinic alpha 7 subunit"/ or "renin angiotensin system"/ or "angiotensin II"/ or "angiotensin I"/ or "Salicylic acid receptors"/ or "angiotensin receptor"/ or "Neuropilin 1"/ or "transferrin receptor"/ or "cysteine proteases"/ or "angiotensin II receptor type 1"/ or "angiotensin II receptor type 1"/ or "nicotinic acetylcholine receptors"/ or "alpha7 nAChRs"/ or "epithelial-mesenchymal transition"/ or "epithelial mesenchymal transition"/ or "receptor tyrosine kinase"/ or "snail family transcription repressor"/ or "Zinc finger E-box binding homeobox 2"))	
	AND	(exp respiratory system/) **OR** ((bronch*/ or alveol*/ or lung/ or nasal/) or ("respiratory tract"))	
OVID search B	-	(exp cigarette smoking/ or exp smoking device/ or exp smoking/ or exp cigar smoking/ or exp vaping associated lung injury/ or exp vaping/) **OR** ((smok*/ or vap*/ or cigarette*/ or e-cigarette*/ or electronic-cigarette*/ or e-cig*/ or nicotine end*/ or electronic cigarette*) or (nicotine/ or tobacco/ or ECIG/ or TCIG/ or ENDS) or ("electronic nicotine delivery system") or (cigarette* adj3 smoke*))	
	AND	(exp virus entry/ or exp cholinergic system/ or exp renin angiotensin aldosterone system/ or exp "angiotensin[1-7]"/ or exp angiotensin converting enzyme 2/ or exp angiotensin/ or exp epithelial mesenchymal transition) **OR** ((ACE2/ or RAAS/ or ACE/ or TMPRSS2/ or TMPRSS4/ or ADAM17/ or CHRNA7/ or RAS/ or ANG-II/ or ANG-I/ or Furin/ or Cathepsin/ or hACE2/ or MasR/ or NRP1/ or TFRC/ or AXL/ or ASGR1/ or KREMEN1/ or CD147/ or TfR/ or IFN-beta1/ or IFNB1/ or CTSL/ or CTSB/ or AGTR2/ or AT2/ or AGTR1/ or AT1/ or MAS1/ or nAChRs/ or EMT/ or LZTFL1/ or SNAI*/ or ZEB2/ or IFI27) or ("angiotensin converting enzyme 2"/ or "renin angiotensin aldosterone system"/ or "renin-angiotensin-aldosterone system"/ or "angiotensin converting enzyme"/ or "transmembrane serine protease 2"/ or "transmembrane serine protease 4"/ or "ADAM metallopeptidase Domain 17"/ or "Nicotinic acid receptor"/ or "cholinergic receptor nicotinic alpha 7 subunit"/ or "renin angiotensin system"/ or "angiotensin II"/ or "angiotensin I"/ or "Salicylic acid receptors"/ or "angiotensin receptor"/ or "Neuropilin 1"/ or "transferrin receptor"/ or "cysteine proteases"/ or "angiotensin II receptor type 1"/ or "angiotensin II receptor type 1"/ or "nicotinic acetylcholine receptors"/ or "alpha7 nAChRs"/ or "epithelial-mesenchymal transition"/ or "epithelial mesenchymal transition"/ or "receptor tyrosine kinase"/ or "snail family transcription repressor"/ or "Zinc finger E-box binding homeobox 2"))	
	AND	(exp respiratory tract infection/ or exp respiratory virus/) **OR** (COVID-19/ or coronavirus*/ or coronovirus*/ or SARS-CoV-2/ or SARS-CoV-1/ or influenza/ or SARS/ or 2019-nCoV/ or HCoV-19/ or MERS/ or RSV/ or SARSCoV1/ or SARSCoV2/ or SARSCoV19/ or H1N1/ or ("novel coronavirus"))	

S1 continued…

Database and search	Joining term	Subject headers **OR** key words (OVID); Key words (WoS)	Total no. results
WoS search A	-	(smok* or vap* or cigarette* or e-cigarette* or electronic-cigarette* or e-cig* or nicotine end* or electronic cigarette*) or (nicotine or tobacco or ECIG or TCIG or ENDS) or ("electronic nicotine delivery system" or "cigarette smoking" or "smoking device" or "cigar smoking" or "vaping associated lung injury" or "vaping") or (cigarette* NEAR/3 smoke*)	6465
	AND	(ACE2 or RAAS or ACE or TMPRSS2 or TMPRSS4 or ADAM17 or CHRNA7 or RAS or ANG-II or ANG-I or Furin or Cathepsin or hACE2 or MasR or NRP1 or TFRC or AXL or ASGR1 or KREMEN1 or CD147 or TfR or IFN-beta1 or IFNB1 or CTSL or CTSB or AGTR2 or AT2 or AGTR1 or AT1 or MAS1 or nAChRs or EMT or LZTFL1 or SNAI* or ZEB2 or IFI27 or angiotensin) or ("angiotensin converting enzyme 2" or "renin angiotensin aldosterone system" or "renin-angiotensin-aldosterone system" or "angiotensin converting enzyme" or "transmembrane serine protease 2" or "transmembrane serine protease 4" or "ADAM metallopeptidase Domain 17" or "Nicotinic acid receptor" or "cholinergic receptor nicotinic alpha 7 subunit" or "renin angiotensin system" or "angiotensin II" or "angiotensin I" or "Salicylic acid receptors" or "angiotensin receptor" or "Neuropilin 1" or "transferrin receptor" or "cysteine proteases" or "angiotensin II receptor type 1" or "angiotensin II receptor type 1" or "nicotinic acetylcholine receptors" or "alpha7 nAChRs" or "epithelial-mesenchymal transition" or "epithelial mesenchymal transition" or "receptor tyrosine kinase" or "snail family transcription repressor" or "Zinc finger E-box binding homeobox 2" or "virus entry" or "cholinergic system" or "angiotensin[1-7]")	
	AND	(bronch*/ or alveol*/ or lung/ or nasal/) or ("respiratory tract" and "respiratory system").	
WoS search B	-	(smok* or vap* or cigarette* or e-cigarette* or electronic-cigarette* or e-cig* or nicotine end* or electronic cigarette*) or (nicotine or tobacco or ECIG or TCIG or ENDS) or ("electronic nicotine delivery system" or "cigarette smoking" or "smoking device" or "cigar smoking" or "vaping associated lung injury" or "vaping") or (cigarette* NEAR/3 smoke*)	
	AND	(ACE2 or RAAS or ACE or TMPRSS2 or TMPRSS4 or ADAM17 or CHRNA7 or RAS or ANG-II or ANG-I or Furin or Cathepsin or hACE2 or MasR or NRP1 or TFRC or AXL or ASGR1 or KREMEN1 or CD147 or TfR or IFN-beta1 or IFNB1 or CTSL or CTSB or AGTR2 or AT2 or AGTR1 or AT1 or MAS1 or nAChRs or EMT or LZTFL1 or SNAI* or ZEB2 or IFI27 or angiotensin) or ("angiotensin converting enzyme 2" or "renin angiotensin aldosterone system" or "renin-angiotensin-aldosterone system" or "angiotensin converting enzyme" or "transmembrane serine protease 2" or "transmembrane serine protease 4" or "ADAM metallopeptidase Domain 17" or "Nicotinic acid receptor" or "cholinergic receptor nicotinic alpha 7 subunit" or "renin angiotensin system" or "angiotensin II" or "angiotensin I" or "Salicylic acid receptors" or "angiotensin receptor" or "Neuropilin 1" or "transferrin receptor" or "cysteine proteases" or "angiotensin II receptor type 1" or "angiotensin II receptor type 1" or "nicotinic acetylcholine receptors" or "alpha7 nAChRs" or "epithelial-mesenchymal transition" or "epithelial mesenchymal transition" or "receptor tyrosine kinase" or "snail family transcription repressor" or "Zinc finger E-box binding homeobox 2" or "virus entry" or "cholinergic system" or "angiotensin[1-7]")	
	AND	(COVID-19 or coronavirus* or coronavirus* or SARS-CoV-2 or SARS-CoV-1 or influenza or SARS or 2019-nCoV or HCoV-19 or MERS or RSV or SARSCoV1 or SARSCoV2 or SARSCoV19 or H1N1) or ("novel coronavirus" or "respiratory tract infection" or "respiratory virus")	

Supplementary information 2: Data extraction summary of the studies selected for inclusion within the literature review.

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Agraval, H., et al., 2022	Cigarette smoke extract	100% CSE (2 x cigarettes in 10mls pre-warmed media) diluted to a variety of concentrations for use (0.5-20%)	Marlboro red cigarettes	Human	Primary small airway epithelial cells	Upregulated following CS exposure: COX-2, MMP2, MMP9	
Albano, G.D., et al., 2018	Cigarette	10 to 20% CSE for 3 cycles per day for 4 days.	Commercially available cigarettes (Malboro Red Label, Phillip Morris International, Switzerland)	Cell line	16HBE		Upregulated following CS exposure: PEBP1, BETA2AR, ERK1/2, MAPK1/2, ERK1/2, MACHRM3, CHAT, ACH, IL8, NOX4
Aliee, H., et al., 2020	SARS-CoV-2 and smoking	Patient smoker or non-smoker	N/A	Human	Nasal brush or curettage and bronchial brush or biopsy	Upregulated following CS exposure: ACE2, TMPRSS2, FURIN, BSG. Downregulated following CS exposure: CTSL.	
Al-Wadei, H.A.N., et al., 2010	NNK tobacco component	1ml for 7 days		Cell line	HPL1D (small airway epithelial)	Upregulated following CS exposure: VEGF. Downregulated following CS exposure: NRP1.	Upregulated following CS exposure: VEGF, CHRNA7.
Andrault, P., et al., 2019	Cigarette condensate	pBECs treated with CSE(0-50%) for 2hrs and 24hrs in 5mL media	3R4F	Human	Primary bronchial epithelial cells	Upregulated following CS exposure: P38, MAPK, CPLA2, CYP1A1	Upregulated following CS exposure: CatS, TOS, TAS
Arredondo, J., et al., 2006	NNK or NNN tobacco component	1uM NNK for 24hrs	N/A	Cell line	BEP2D bronchial cells	Upregulated following exposure to CS: PCNA, BCL-2, NF-KB, GATA-3, STAT1.	

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Basil, M. et al., 2022.	Cigarette smoke	60mins twice/d for 6 months.	1R6F research cigarettes	Human and Ferret	AT2 cells; Lung.	Upregulated following CS exposure: SCGB3A2, LAMP3	
Blank, U., et al., 1997	Nicotine	0.5 - 3.0mM nicotine	N/A	Human	Nasal epithelial cells	Upregulated following nicotine exposure: Ca2+.	
Cai, G., et al., 2020	Smoking	Non-smoker or smoker	N/A	Human	Lung airway epithelium	Upregulated following CS exposure: ACE2, FURIN	
Carlier, F. et al., 2021	Smoker	Smoker vs non-smoker samples		Human	Primary human bronchial epithelial cells	Upregulated following CS exposure: SPDEF, FOXA3, IL-8, CXCL-8. Downregulated following CS exposure: MYB, FOXJ1, DNAI1	Downregulated following CS exposure: occludin
Carlisle, D.L., et al., 2004	Cigarette smoke and nicotine	Never smoker, ex-smoker and active smoker	N/A	Cell line and Human	Bronchial epithelial cells and BEAS2B cell line	Upregulated following CS exposure: CHRNA1, CHRNA5. Upregulated following nicotine exposure: CA2+, PKC, P38. Downregulated following CS exposure: CHRNB4, CHRNA7, CHRNB2, CHRNAGAMMA.	Upregulated following CS exposure: CHRNA1, CHRNA5, CHRNB2, CHRNAGAMMA.
Chakladar, J., et al., 2020	Smoking	Smokers vs former smokers	N/A	Human	Lung and oral epithelium	Upregulated following CS exposure: ACE2, TMPRSS2, ADAM17, ANDROGEN RECEPTOR	
Chen, P., et al., 2021.	Smoking	CSE 1-4% for 24h. 100% CSE is 10 cigarettes bubbled into 25ml media.	Suyan, Jiangsu Cigarettes, China	Cell line and human	Lung tissue and 16HBE cells	Upregulated following CS exposure: EGR3, BAX, CASPASE-3, BCL-2, IL6, TNFALPHA, COX2, XIST	

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Chen, Q., et al., 2021	SARS-CoV-2			Mice	Humanised ACE2 mice via CRISPR/Cas9	Upregulated following SARS-CoV-2 exposure: IL-1alpha, IL-2, IL-5, IL-6, IL-17A, IL28, IL-22, IP-10, MCP-1, MCP-3, Eotaxin, IFNgamma.	
Chen, Q., et al., 2022	SARS-CoV-2	Victorian Infectious Diseases Reference strain at an MOI of 0.05, 0.1 or 1 for 1h at 37 degrees Celsius		Human	Primary human bronchial epithelial cells; Bci and BEAS2b cell line		Upregulated following SARS-CoV-2 exposure: ZO-1
Chu, M., et al., 2005	Nicotine	0.5uM nicotine supplemented into media for a constant concentration and exposure for 8 wks	N/A	Mouse	Lung epithelial cells (LA4)		Upregulated following nicotine exposure: Ras, EGFR, Raf, P13K, cyclin D1.
Chung, S., et al., 2015	Smoking	60mg/m^3 6h/d for 5 days	3R4F Kentucky	Human and Mice	Airway epithelial cells and 129/Sv mice	Upregulated following CS exposure: nSMase2, SRC, MAPK, P38.	
Chung, S., et al., 2019	E-cig with or without nicotine and nicotine alone	Vapour containing 36mg/ml nicotine for 24h or 1uM nicotine	Joytech VTC mini (SciReq, Montreal Canada)	Human and sheep	Primary bronchial epithelial cells and tracheal secretions	Upregulated following nicotine exposure: TRAP1	
Cortijo, J., et al., 2011	Cigarette and nicotine	Up to 30% CSE (10%, for 24hr). Direct exposure to nicotine (10uM)	-	Human	Bronchial lung tissue	Upregulated following CS exposure: MUC5AC, EGFR, P42/P44, M3	Upregulated following CS exposure: MUC5AC, M3
Daniell, H., et al., 2022	SARS-CoV-2	Infected patients		Human	Lung autopsy		

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Di Vincenzo, S. et al., 2022	Whole cigarette smoke	4-5 mins CS exposure with 10 min ventilation	3R4F Cigarettes	Human	Primary bronchial epithelial cells	Upregulated following CS exposure: SNAIL1, ZEB1, SNAIL2. Downregulated following CS exposure: CDH1, VIMENTIN, NOTCH1, E-CADHERIN, MMP9, TP63, SCGB1A1, JAG1, HES1.	Downregulated following CS exposure: E-CADHERIN, VIMENTIN.
Downs, C., et al., 2011	Cigarette	1, 10, 50, 100% CSE for 24hrs		Rat	Alveolar epithelia type 1 cells	Upregulated following CS exposure: ERCC6, ZMYND17, NQO1. Downregulated following CS exposure: APOE, CYGB, PRNP, SLC38A1, TMOD1	
Du, H., et al., 2012	Cigarette	10 mins every other day (1,5 or 20% smoke concentration)	Xuzhou cigarette factory, China (contain 11mg tar oil and 0.8mg nicotine)	Cell line	BEAS-2B	Upregulated following CS exposure: Survivin. Downregulated following CS exposure: MGMT, RASSF1A.	Downregulated following CS exposure: Caspase-3
Ebrahimpour, A., et al., 2019	Nicotine	1nM, 10nM and 100nM for 24h	N/A	Human	Primary epithelial cells	Upregulated following nicotine exposure: TNFRSF1B, TNFRSF4, TNF-ALPHA, NFKB, ILIBETAIL2RB, IL17B, IL21 RECEPTOR, TWIST1, CHRNA7, ALPHA7 NACHR	
Eurlings, I., et al., 2014	Cigarette	BEAS-2B 1% CSE for 15 mins twice every 24hrs. Mice were exposed 4 times per day, 5 days a week for 24 weeks	Filters were removed from 3R4F Research cigarettes	Cell line and mice	BEAS-2B	Upregulated following CS exposure: PAL1, vimentin, collagen, CA9, HIf-1ALPHA. Downregulated following CS exposure: E-cadherin, keratin18.	Upregulated following CS exposure: fibronectin, vimentin. Downregulated following CS exposure: E-CADHERIN

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Foronjy, R., et al., 2016	Cigarette smoke; Non-smoker and smoker	Mice 4h/d, 5d/wk for 6 months; Humans 35ml puffs and 6 puffs/cigarette for 4, 8 or 12 cigarettes	3R4F Kentucky	Mice and human	TLR9-/- mice and PTPLB-/- mice; normal human bronchial epithelial cells.	Upregulated following CS exposure: TLR9, CXCL5, G-CSF, MMP2, IL6, IL1BETA	Upregulated following CS exposure: TLR9
Fu, X., et al., 2009	Nicotine	1-500uM	N/A	NHP	Bronchial epithelial cells	Upregulated following nicotine exposure: nAChR.	
Gahring, L.C., et al., 2017	Cigarette	25-50 cigarettes for 225mins, 5d/wk for 4 months	3R4F Kentucky	Mice	α7G (CHRNA7 GFP tagged reporter) and α7E260A:G (homozygous receptor diminishes calcium current by 90% affecting developmental phenotypes and inflammatory responses)	Upregulated following CS exposure: SCGB1A1, ACTLB, CTSD, CTSS, VIM, CD74. Downregulated following CS exposure: CYP2F2	
Gao, H., et al., 2020	Smoke component BAP	48h 5ul/ml		Cell line	BEAS-2B	Upregulated following exposure to BaP: AhR, HSP90AA1, ARNT, CYP1A1, HIF-1ALPHA, TWIST1, TWIST2, SNAI2, BETA-CATENIN, N-CADHERIN, FIBRONECTIN, VIMENTIN, K-RAS. Downregulated following exposure to BaP: NRF2, E-CADHERIN.	

S2 continued…

Reference	Exposure		Model			Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
*Gebel, S., et al., 2006	Cigarette	300 or 600TPM/I 1h, twice/d, 5d/wk for 2, 7 or 13 wks	2R4F Kentucky	Rat	Lung	Upregulated following CS exposure: MT1a, MT2, FLAVIN, NR1D2, NQO1, ALDH3A1, CYP1A1, CXCL1, C3, DMBT1, MYBL1, FKBP38. Downregulated following CS exposure: ZMYND11, SPP1	Upregulated following CS exposure: Chitinase, NQO1, ALDH-3, EROD.
Geraghty, P., et al., 2014.	Cigarette	4hrs per day, 5 days a week for 1 day, 1 month, 2 months or a year		Mice and human	Human small epithelial cells	Upregulated following CS exposure: Src, c-Src, c-raf, MMP9, IL1BETA, IL6, TNF-ALPHA, MCP-1, CATHEPSINK, MMP12, MMP9, MMP12	Upregulated following CS exposure: P-ERK, P-JNK, P-P38
Ghosh, A., et al., 2021	Cigarette and pseudovirus	HBECs exposed to 14 puffs a day for either 1(acute) or 4 days(chronic). 3x10^4 U/ml pseudovirus. Varying concentrations of CSC (21.6-176uM nicotine conc) or JUUL tobacco-flavoured e-liquid (364-3,641uM nicotine conc) for 24hrs and infected with 2.5-5x10^5 U/ml pseudovirus.	1R6F	Human	Primary human bronchial epithelial cells; primary tracheobronchial cells and small airway epithelial cells	Upregulated following CS exposure: ACE2. Upregulated following SARS-CoV-2: ACE2.	Upregulated following CS exposure: ACE2.
Ginzkey, C., et al., 2012	Nicotine	0.001, 0.01, 0.1, 1.0, and 4.0mM		Human and cell line	Primary nasal mucosa and BEAS-2B cell line		

S2 continued…

Reference	Exposure		Model			Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Golovatch, P., et al., 2009	Cigarette smoke	2 x 70ml puffs for whole body exposure 4h/d, 5d/wk for 1-12 wks	Kentucky 2RF reference cigarette	Guinea Pigs	Lung epithelial cells	Upregulated following CS exposure: JNK2, JNK1	Upregulated following CS exposure: P-ERK1/2, P-JNK1, P-JNK2, MAPK, Cathepsin K, MMP9. Downregulated following CS exposure: Elastin, collagen.
Gundavarapu, S., et al., 2012	Nicotine	6h/d, 5d/wk for 2wks. 1.5mg tpm.	N/A	Human	Normal bronchial epithelial cells	Upregulated following nicotine exposure: MUC5AC.	Upregulated following nicotine exposure: MUC5AC.
Guo, J., et al., 2005	Nicotine	0.5uM nicotine for various time periods	N/A	Rat	Lung epithelial cells		Upregulated following exposure to nicotine: Ras, cyclinD1, PKC, ROS, P13K, AKT
Haslbauer, J., et al., 2022	SARS-CoV-2	Infected patients		Human	Primary post mortem biopsy		Downregulated following SARS-CoV-2 exposure: AGTR1.
Hirschi-Budge, K. et al., 2022	Second-hand smoke exposure	The 10s primary puff is cleared and expelled so that the mice are only exposed to the side stream smoke three times/ 20 mins, 5d/wk	3R4F Cigarettes	Mice	C57BL/6 WT, RAGE knockout and transgenic	Upregulated following CS exposure: RAGE, Ras, NF-KB	Upregulated following CS exposure: RAGE, Ras, NF-kB, Cl-Caspase-3.
Ho, Y., et al., 2005	NNK tobacco component	Human cells 0-5uM; Mice 9.1mg	N/A	Human and mice	Normal human bronchial epithelial cells and small airway epithelial cells	Upregulated following CS exposure: CyclinD1, NFKB	Upregulated following CS exposure: CyclinD1, NFKB, P-ERK1/2, P38, CD1LUC, IKBALPHA, P-IKBALPHA, NFKB

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Hou, W., et al., 2020	Cigarette smoke	CSE (10ug/ml, 20ug/ml and 50ug/ml) was introduced into the chip media for 8d and cells recovered in the absence of CSE for 7d		Cell line	BEAS2B	Upregulated following CS exposure: IL6, TNF-ALPHA, CLDN7, CLDN8. Downregulated following CS exposure: CLDN1, OCLD, BETA CATENIN, CDH1.	Upregulated following CS exposure: PHOSPHORYLATED STAT3, PHOSPHORYLATED ERK, C-MYC, CYCLIND1. Downregulated following CS exposure: E-CADHERIN, BETA CATENIN.
Hudlikar, R., et al., 2022.	NNK	24uMol/0.1ml		Mice	A/J female	Upregulated following CS exposure: NKRF, SOX14, CES3A, CYP3A25, UGT2B36, UGT2B5, CYP2J5, HRG, AKR1D1, BC024386, CCYP2C50, CYP2C54, JPH2. Downregulated following CS exposure: TRPC7, PDK2, ATXN1, SDR39U1, MPHOSPH8, FRAT1, TADA2A, TRAPPC4, CDK9, UKL1, NRARP.	
Hung, Y., et al., 2016	Cigarette (0.8mg nicotine)	CS 4 times/d for 1-3 weeks		Mice	Lung tissue		Upregulated following CS exosure: TGFBETA1, TNF-ALPHA, IL6, ACE, ACE2, P-P38, P-JNK, P-ERK1/2. Downregulated following CS exposure: MMP2, MMP9.
Irie, H., et al., 2022	Cigarette	60min/d, 5d/wk for 3wk	Malboro (12mg tar/ 1mg nicotine)	Mice	C57BL6J; GFP and surfactant protein C expressing mice	Upregulated following CS exposure: GPX1, GCLM, GCLC.	Upregulated following CS exposure: CPT1A.

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Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Irie, H., et al., 2022	CS with nicotine	Mice 60min/d, 5d/wk	Malboro (12mg tar/1.0mg nicotine	Mice	C57BL6J mice and mice expressing GFP in surfactant protein C	Upregulated following CS exposure: CPT1A.	
Izzotti, A., et al., 2010	Environmental cigarette smoke	28 days		Rats	Lungs	Downregulated following CS exposure: NF-KBETA, P53, TGF-BETA, ELK-1, FOS	
Jiang, J.-X., 2017	Cigarette smoke	10 cigarettes, 1.5hr whole body exposure	Kentucky 3R4F reference cigarette	Cell line and mice	16HBE and C57BL/6 mice	Upregulated following CS exposure: IL6, IL8, RAC1	Upregulated following CS exposure: RAC1, IL6, IL8, CD68, CD11B, ERK1/2, STAT3
Lallai, V., Manca, L. and Fowler, C., 2021	E-cigarette and nicotine	1 puff every 5 mins for an hour for 5 days with 7.5mg/mL nicotine or without		Mice	C57BL/6 J WT lungs	Upregulated following e-cig with nicotine: ACE2. Downregulated following e-cig with nicotine: alpha5 nAChR.	Upregulated following e-cig with nicotine: ACE2.
Lam, D., et al., 2016	Nicotine	Never smoker, active smoker or ex-smoker. 100uM nicotine.	N/A	Human and cell line	Human bronchial epithelial cells and HBEC-KT 2-5 cell lines	Upregulated following nicotine exposure: CHRNA5, CHRNA7, CHRNA6. Downregulated following CS exposure: CHRNA6.	
Latha, M., et al., 1991	Cigarette smoke	Exposed twice/d for 90d for 15mins(day 1), 30mins(day 2), 45mins(day 3) and 1hr(day 4+)		Rats	Lungs	Upregulated following CS exposure: Glycosaminoglycans, CTSD, PARG, GUSB	
Lee, A., et al., 2020	Smoking	Current smoker or former smoker	N/A	Human		Upregulated following CS exposure: ACE2	

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Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Lee, H.S. and J. Kim, 2013	Cigarette condensate	2 cigarettes smoked consecutively over 10 minutes bubbled through 10mL media and CSE was used at 0.3-300ug/ml	2R4F	Human	Primary nasal epithelial cells		
Lee, I., et al., 2020	Cigarette / SARS-CoV-2	Former, current or never smoker	-	Human and mice	Human nasal tissue, lung tissue	Upregulated following SARS-CoV-2 exposure. No significant difference in ACE2 or TMPRSS2 following CS exposure.	
Lemjabbar, H., et al., 2003	Smoke exposures	1mg smoke particulate per ml		Mice and Human	C57 mice; primary human airway epithelial cells		Upregulated following CS exposure: P-EGFR. Downregulated following CS exposure: ADAM17.
Leung, J., et al., 2020	Cigarette	Current, former or never smoker samples	N/A	Human	Primary bronchial epithelial cells	Upregulated following exposure to CS: CHRNA7, ACE2	
Li, D., et al., 2022	SARS-CoV-2 pseudovirus	Transocular inoculation of mice		Mice	ACE2 dependence, humanised ACE2 and ACE2 KO mice	Upregulated following SARS-CoV-2 exposure: ACE2.	
Li, E., et al., 2014	Cigarette	CSE(1-100% exposure) for varying lengths of time (1 hr - 8 wks).		Human	Primary bronchial epithelial cells adjacent to a cancer tumour		Upregulated following CS exposure: VIMENTIN, GRP78, IRE1ALPHA, P13K/AKT, MTOR, IKKALPHA, NF-KB, CYCLIND1. Downregulated following CS exposure: E-CADHERIN.

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Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Li, F., et al., 2012	Smoking	7 cigarettes (day1), 9 cigarettes (day 2) and 11 cigarettes (day 3 and 4).	3R4F Kentucky	Mice	C57BL/6	Upregulated following CS exposure: Shp2, IL8.	Upregulated following CS exposure: Shp2, IL8, MMP2, KC
Li, F., et al., 2021	SARS-CoV-2 pseudovirus	MOI of 5 for 24h		Human cell line; Mice	16HBE; BALB/c mice		Upregulated following SARS-CoV-2 exposure: LC3-II, BAX, IL6, IL8, TNF-ALPHA. Downregulated following SARS-CoV-2 exposure: p62, BCL-2, mTOR, AKT.
Li, G., et al., 2020	Smoking and SARS	12, 24 and 48h post SARS infection; before and after smoking 3 cigarettes in 24h		Human	Lung tissue, BAL, bronchial epithelial cells, small epithelial cells and SARS infected cells	Upregulated following CS exposure: ACE2. Upregulated following SARS-CoV-2 exposure: ACE2, IL6, IL10, IL1.	
Li, H., et al., 2021	Cigarette	Smoker vs non-smoker - CSE exposure for 48hrs	N/A	Human	Bronchial epithelial cells	Upregulated following CS exposure: TROP2, P38, P65, IL6, IL8, VIMENTIN. Downregulated following CS exposure: E-CADHEIRIN	Upregulated following CS exposure: TROP2, P38
Li, Q., et al., 2010	Cigarette smoke	10 cigarettes, 10 puffs/cigarette, 1 puff/min into 20mls buffer	Camel cigarettes	Cell line	BEAS2B	Upregulated following CS exposure: ROS, MUC5AC	Upregulated following CS exposure: MUC5AC, SRC, EGFR, P-ERK, JNK
Li, Q., et al., 2011	Cigarette smoke and Nicotine	100ug/ml CSE or 20uM nicotine for 24h	Camel brand cigarette	Cell line	HBE16	Upregulated following CS exposure: MyD88. Downregulated following nicotine exposure: TNF-ALPHA.	Upregulated following CS exposure: MyD88, 1-Kbalpha, P65. Downregulated following nicotine exposure: TNF-ALPHA, P65.

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Reference	Exposure		Model			Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Li, Q., et al., 2012	Cigarette and nicotine	CS for 12hr; Nicotine conc 5, 10, 20uM	Camel brand	Cell line	HBE16	Upregulated following nicotine exposure: CHRNA1, CHRNA5, CHRNA7, CHRNB2, TNF-ALPHA, IL8, IL6.	Upregulated following nicotine exposure: CHRNA1, CHRNA5, CHRNA7, TNF-ALPHA, IL6, IL8.
Li, W., et al., 2021	Smoking	Smoker vs Non-smoker	N/A	Human	Nasal mucosa, bronchial mucosa and pulmonary alveoli	Downregulated following CS exposure: ACE2	Downregulated following CS exposure: ACE2
Lin, C., et al., 2021	Cigarette	Current, former or never smoker samples	N/A	Human	AT2 cells from donors	Upregulated following CS exposure: CD147, HERPUD1, GRP78, CD209L, ACE2, TMPRSS2,	Upregulated following CS exposure: CD147, HERPUD1, GRP78, CD209L, ACE2, TMPRSS2
Liu, A., et al., 2021	Cigarette smoke	Non-smoker, current smoker and ex-smoker; Mice exposed to 5 cigarettes, 4 times per day, 6 days/week for 1, 4 or 8 months.	Unknown but 14mg tar and 1mg of nicotine per cigarette	Human and mice	Small epithelial cells and type II alveolar cells	Upregulated following CS exposure: HIF-1alpha, iNOS, 4HNE, ACE2	Upregulated following CS exposure: ACE2. Downregulated following CS exposure: ACE2.
Lupacchini, L., et al., 2020	Nicotine	1x10^7M nicotine for 1h or 48h	N/A	Human	Bronchial epithelial cells		Upregulated following nicotine exposure: EGFR, PHOSPHO EGFR, PHOSPHO38, VEGF, VIMENTIN, FIBRONECTIN, VEGF, KI67, CA2+, ACE2, ALPHA7 NACHR. Downregulated following nicotine exposure: ATP, P53, PHOSPHO P53, E-CADHERIN.

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Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Lv, J., et al., 2021	SARS-CoV-2	Increased viral load and measured infection at 2hr or 72hr and control (no infection)		Cell line, murine and NHP	BEAS-2B and murine alveolar epithelial type II cells	Upregulated following SARS-CoV-2 exposure: AhR, ACE2.	
Ma, L., et al., 2020	Cigarette	24h at 20%CSE		Mice	Bronchial epithelial cells	Upregulated following CS exposure: IL17R, VIMENTIN. Downregulation following CS exposure: E-CADHERIN	Upregulated following CS exposure: NF-KB
Maehira, F., et al., 1999	Cigarette	Via ash tray 10cm below rat cage for 1hr twice/d for 8, 12 or 20 wks		Rat	lungs	Upregulated following CS exposure: KRAS, C-PKC. Downregulated following CS exposure: PKC, C-PKC.	Upregulated following CS exposure: 8OHDG. Downregulated following CS exposure: KINASEA
Mao, Y. and Feng, H., 2022	CSC	5, 10 or 20% cigarette smoke concentrations	15 lit cigarettes (Sichuan China Tobacco Industry Co., Ltd.)	Human	16-HBE cell line		Upregulated following CS exposure: slug, alpha DMA, collagen IV, FN1, TGF-beta1, p-SMAD3. Downregulated following CS exposure: e-cadherin, CC16.

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Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Marshall, K., et al., 2020	E-cig and nicotine	e-cig aerosol (18mg/mL nicotine) exposure for 8 months		Mice	Lungs	Upregulated following CS exposure: BCL-XL. Upregulated following e-cig with nicotine exposure: BCL-XL. Downregulated following CS exposure: E-CADHERIN, CRM1. Downregulated following e-cig with nicotine exposure: E-CADHERIN, CRM1.	Upregulated following CS exposure: CYP1A1, CYP2A5, AhR, NRF2, SOD1, BCL-XL, P21. Upregulated following e-cig with nicotine exposure: CYP1A1, CYP2A5, AhR, NRF2, SOD1, BCL-XL. Downregulated following CS exposure: E-CADHERIN, CRM1. Downregulated following e-cig with nicotine exposure: E-CADHERIN, CRM1.
Martinez-Garcia, E., et al., 2008	Nicotine	1 to 500uM nicotine daily for 7 days		Human	Normal bronchial epithelial cells	Upregulated following nicotine exposure: N-CAM, Nef-M, Pax-3, alpha7 nAChR. Downregulated following nicotine exposure: e-cadherin, ZO-1, beta2 nAChR.	Upregulated following nicotine exposure: EGFR.
Masso-Silva, J., et al., 2021	E-cig and nicotine	Vape pen (24mg/ml with nicotine) / box (with or without 6mg/ml nicotine) 60min/d for 3-6 months; JUUL exposure (with 59mg/ml nicotine)20 mins 3 times/d for 1 month		Mice	Lungs	Upregulated following e-cig with nicotine exposure: KRT83. Downregulated following e-cig with nicotine exposure: CD177, FACMR, TLR9, FCGRL, CCR2, IL6, IL1, IL2, TNF, IFNGAMMA, TGFBETA, P13K, JAK/STAT, ERK1, ERK2, IKB, NFKB, CD40, ACE2, AGT, SLC7.	

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Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
McAlinden, K., et al., 2021	E-cig with or without nicotine, PG:VG or CSE	E-cig condensate (0.05%, 0.1%, 0.5% or 1% conc) with or without nicotine (18mg/mL or 60mg/mL); CSE (0.5% or 1% conc).	Watermelon and menthol flavoured e-liquid(Juicius Maximus, VapeTrail); 1R6F Kentucky Tobacco Research, USA	Human	BEAS-2B cell line and primary small airway epithelial cells	Upregulated following CS exposure: ACE2. Upregulated following e-cig with nicotine exposure: ACE2.	Upregulated following CS exposure: ACE2. Upregulated following e-cig with nicotine exposure: ACE2.
Mebratu, Y., et al., 2011	Cigarette	Mice exposed to 250mg/m^3 CS for 6h/d, 5d/wk for 3 wks		Human and Mice	Primary airway epithelial cells, ; Mice C57BL/6, Mouse airway epithelial cells	Upregulated following CS exposure: MUC5AC. Downregulated following CS exposure: BIK	Upregulated following CS exposure: STAT1, ERK1/2
Mishra, R., et al., 2016	Cigarette	Cells CSE exposed for 10 minutes; Mice 6h/day, 5d/wk	2R4F	Human and mice	Primary alveolar epithelial cells; NuLi-1 cells (nontransformed human airway epithelial cell line and normal human bronchial epithelial) Mice C57B16/J.		Upregulated following CS exposure: HER2, PHER2, HER3, pHER3, pEGFR, EGFR, IL6.
Monzon, M., et al., 2011.	Cigarette	Smoker or non-smoker	N/A	Human	Normal bronchial epithelial and tracheobronchial		Upregulated following CS exposure: MUC5AC, MUC5B, MCP-1, CCR2B

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Reference	Exposure		Model			Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Nakayama, T., et al., 2021	Cigarette and SARS-CoV-2	CSE 1hr/d for 3d before SARS-CoV-2 inoculation; Viral infection exposure involved 100ul of viral suspension 5-10x10^5PFU/mL for 2hrs	3R4F University of Kentucky (9.4mg tar/ 0.726mg nicotine)	Human	Nasal epithelial cells and COVID-19 infected patient samples		Upregulated following CS exposure: IFNB1.
Nath, S., et al., 2019	Cigarette	4h/d, 5d/wk	3R4F Cigarettes	Mice	C57BL/6J CTSS knockout mice	Upregulated following CS exposure: CTSE, CTSS, CTSG.	Upregulation following CS exposure: CTSS
O'Donnell, R. et al., 2004	Smoker vs non-smoker tissues			Human	Bronchoscopy tissues		Upregulated following CS exposure: EGFR, ERBB3, MUC5AC.
Ogawa, F., et al., 2019	Cigarette smoke	CSE from one cigarette bubbled through 12.5ml media	Malboro Red cigarette	Human	Non-smoker primary basal cells	Upregulated following CS exposure: KRAS activation, RAS	Upregulated following CS exposure: KRAS, RAS
Osborne, J. et al., 2014	Nicotine	Increasing conc for 24-48h	N/A	Human cell line	HBEC3KT-53; HBEC3KT, HBEC4KT, HBEC30KT	Upregulated following nicotine exposure: NEUROD1, CHRNA3, CHRNA5, CHRNA7, CHRNB2, CHRNB4.	Upregulated following CS exposure: NEUROD1. Downregulated following CS exposure: p53.
Peng, Y., et al., 2022	Smoker	Smoker vs non-smoker samples		Human	Primary small airway epithelial cells	Upregulated following CS exposure: ACE2, TMPRSS2. Downregulated following CS exposure: BSG, ITGAV.	

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Reference	Exposure		Model			Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Phandthong, R., et al., 2022	E-cig	Vitrocell: 1 puff of PBS nicotine (0.3 or 0.003mg/ml) during a 1.5 mins aerosol generation followed by 3 min aerosol deposition. CULTEX: 55ml air or e-cig aerosol for a 4s puff with a 30s interval	JUUL aerosols	Cell line	BEAS-2B and HEK293T		Upregulated following nicotine exposure: ACE2, TMPRSS2. Upregulated following e-cig with nicotine exposure: ACE2, TMPRSS2. Upregulated with e-cig: ACE2. Downregulated with e-cig: TMPRSS2.
Podowski, M., et al., 2012	Cigarette	Mice exposed 2h/day, 5 d/wk, 6/7 wks and MLE12 cells treated with CSE for 72hrs		Mice	Lung tissue	Upregulated following CS exposure: MMP9, MMP12. Downregulation following CS exposure: MAPK, AKT, P38, JNK	Upregulation following CS exposure: TGF-BETA, SMAD2, CTGF
*Polk, W., 2012	Cigarette	CSC from 20 cigarettes with final conc 40mg/mL	3R4F Kentucky Reference	Cell line	BEAS2B	Upregulated following CS exposure: MMP3, MAP1B. Downregulated following CS exposure: KRT7, KRT9, SNAI3, MMP2	Upregulated following CS exposure: BETA-CATENIN, E-CADHERIN
Polosukhin, V., et al., 2011	Cigarette	Current smoker or never smoker	N/A	Human	Lung tissue and bronchial epithelial cells	Upregulated following exposure to CS: MUC5AC, CA IX	Upregulated following exposure to CS: MUC5AC, ERK, HIF-1ALPHA, P-ERK1/2
Profita, M., et al., 2011	Cigarette	18h with 10% CSE	Commercial cigarettes (Marlboro; Philip Morris USA, Richmond, VA)	Cell line	16-HBE	Upregulated following exposure to CS: ChAT, ACh, LTB4, M2, M3	Upregulated following exposure to CS: ChAT, ACh, LTB4, M2, M3

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Qiao, Y., et al., 2021	Cigarette smoke	Smoker vs non-smoker	N/A	Human and mice	Lung tissues		Upregulated following CS exposure: ACE2
Rangasamy, T., et al., 2009	Cigarette	Mice exposed to CS 7 hrs/d, for 1 day(Group II), 8 days (Group III), 1.5 months (Group IV) or 6 months (Group V)	2R4F (2.45mg nicotine per cigarette)	Mice	A/J mice	Upregulated following CS exposure: CYP1A1, CYPB1B1, ADAM8, CTSB, CTSK, MAP2K6.	
Rayner, R., et al., 2022	Smoking and e-cigarette	100ul exposure of whole-smoke conditioned media/e-liquid aerosol conditioned media at various nicotine concentrations for 1h/day for 10 days	3R4F cigarette and commercially available e-liquid 2ith 1.8% nicotine	Human	Primary human bronchial epithelial cells	Upregulated following CS exposure: CYP1A1, CYP1B1, ALDH3A1, AKR1C1, AKR1C2, GXP2, NQO1, FTL, ADH7, CEACAM5, MUCL1, MUC5AC, KRT13, LUCAT1, OSGIN1, MAGEA6, ABCB6, SLC9A5, IL36A. Upregulated following e-cig with nicotine exposure: CYP1B1, MAGEA6, ABCB6, SLC9A5. Downregulated following CS exposure: EN1, CXCL10, CXCL5, CSF3, CCL20.	
Russo, P., et al., 2020	Nicotine	1 x 10^-7M (concentration present in alveolar lining fluids after one cigarette is between 6x10^-6 and 6x10^-5) for 1hr or every 58 hrs for 16 passages.		Human and cell line	Primary bronchial epithelial cells and HBE16	Upregulated following nicotine exposure: ACE2, PHOSPHO-S6 RIBOSOMAL PROTEIN, AKT1, PHOSPHO-AKT, PHOSPHO-P44, P43, MAPK	

S2 continued…

Reference	Exposure		Model			Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Sarkar, P. and Hayes, B., 2007	Tobacco - acrolein	Incubated for 72h with different acrolein concentrations (0-45uM)	N/A	Rat	Lung epithelial cells	Upregulated following CS exposure: COX-2, NF-KB, CA+2, ERK, MEKK	Upregulated following CS exposure: RAF-1
Shaykhiev, R., et al., 2013	Cigarette	Smoker or non-smoker; ALI cells exposed to 1-2% CSE for 24hrs every other day for 2 wks.	Malboro Red cigarette	Human	Large airway epithelial cells	Upregulated following exposure to CS: ERBB4, EGF, NRF-2, KRT6, KRT14, IVL, SFN, CD44, SNA12, VIMENTIN. Downregulated following exposure to: EGFR, ERBB2, TGF-ALPHA, KRT5, FOXJ1, DNAI1, MUC5B, SCGB1A1, E-CADHERIN, TJP1, CLDN, OCLN, PARD3, PARD6, PTEN	Upregulated following CS exposure: EGF. Downregulated following CS exposure: EGFR.
Shen, H., et al., 2022	CS with nicotine	16HBE CSE exposure length unknown; Mice exposed to 10 cigarettes/d for 20d	3R4F Kentucky University	Human and mice	16HBE cell line; C57BL/6 mice	Upregulated following CS exposure: mAChRM3, ChAT, VIM, alphaSMA, Akt, p-Akt. Downregulated following CS exposure: E cadherin.	Upregulated following CS exposure: ChAT, Ach, VIM, alphaSMA. Downregulated following CS exposure: E cadherin.
Sompel, K., et al., 2022	CSC	HBEC cells after 24hr 5ug/ml of CSC, 2/wk for 1-24 weeks; Mice whole body exposure for 6h/d, 5d/wk for 6wks		Human and mice	HBEC3KT and HBEC2KT cells; FZD9 -/- and FVBN mice	Upregulated following CS exposure: VIM. Downregulated following CS exposure: FZD9, ECAD, PPARgamma, PPRE	Upregulated following CS exposure: VIM. Downregulated following CS exposure: ECAD

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Staudt, M., et al., 2018	E-cig and nicotine	10 puffs, wait 30m before another 10 puffs		Humans	Small airway epithelium	Upregulated following e-cig with nicotine exposure: LINC01186, AJUBA, LATS2, AMOTL2, SERPINB2, CST6, RND3. Upregulated following e-cig: LTB4R2, ISG20, GRHL3, TXK, LGALS7B, SLC5A8, BATF3. Downregulated following e-cig with nicotine exposure: BEST3, MYL5, MTIX, SGK1, MT2A, NRIDI, NEURL3. Downregulated following e-cig exposure: DNASEIL2, TMEM220-ASI, TMEM171, PLXDC1, CALML5.	
Tan, L., et al., 2018	Cigarette	Smokers smoker 10-20 cigarettes/d for 3 years; Mice CS exposure 2hrs/d for 3 wks.	3R4F (University of Kentucky)	Human and mice	ATII cells from organ donors; C57BL/6 mice	Upregulated following CS exposure: ADAMTSL4	Upregulated following CS exposure: MMP9, CD147, ADAMTSL4. Downregulated following CS exposure: P18 NFE2, P45 NFE2.
Tang, X., et al., 2021	Cigarette	Human cells: 2.5% CSE for 24h; Rats: 15 cigarettes, 30 min each twice/d, 6d/wk for 16wk	Daqianmen cigarettes (10mg tar, 1mg nicotine and 12mg CO)	Human and Rats	BEAS-2B cell line; Sprague-Dawley rats		Upregulated following CS exposure: IL6, TNFALPHA, MDA, IRON, TFR1. Downregulated following CS exposure: GSH, SLC7A11, GPX4, FTH1.

S2 continued…

Reference	Exposure		Model			Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Tomchaney, M., et al., 2020	Smoking and SARS-CoV-2	Human lung specimen: smoker or non-smoker; Mice: 2h/d, once every 6d for 1-6 months; Primary HBEC: CSE and 5min cigarette smoke and infected with SARS-CoV-2 at an MOI of 0.05 PFU/cell.	3R4F Cigarettes	Human	Lung specimens from smokers without COPD and non-smokers; C57BL/6 mice; Primary human bronchial epithelial cells		Upregulated following CS exposure: GammaH2AX. Downregulated following CS exposure: ACE2, MUC5AC.
Tsutsumi, A., et al., 2020	Cigarette	CS via nasal inhalation 60min/d, 5d/wk for 12 wks.	Malboro (12mg tar/ 1mg nicotine)	Mice	C57BL6 mice	Upregulated following CS exposure: DBP, CFB, NR1D1, VNN1, LY6I, STEAP4, PIGR, NR1D2, TNFRS19, ST6GALNAC3. Downregulated following CS exposure: RETNLA, NPAS2, ARNTL, HMGCS2, CCNJ1, CLCA1, AVPR1A, DEFB5, RASL11A, KLH13.	
*Van der Toorn, M., et al., 2017	Cigarette	Continuous exposure for 12 wks (Low (7.5ug/mL TPM; 2.5uM nicotine), medium (37.5ug/mL TPM; 6uM nicotine) or high (150ug/mL; 24uM nicotine)). Note nicotine concentration much lower in THS compared to 3R4F.	3R4F (University of Kentucky) and tobacco heating system 2.2	Cell line and human	BEAS-2B and primary normal bronchial epithelial cells	Upregulated following exposure to CS: IL1A, IL8, CXCL8, CSF-2, CSF-3, VEGFA, MMP1, MMP9, IL6 TIMP1. Downregulated following exposure to: CCL5, CXCL10, TIMP2	Upregulated following CS exposure: vimentim, mBCL, PH2AX. Downregulated following CS exposure: e-cadherin, DHE.

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
*Veljkovic, E., et al., 2011	Cigarette	20ug/ml of CSC (0.002 cigarettes/ml)		Cell line	BEAS-2B	Upregulated following CS exposure: PAK3, ADAM19, AGTR1, TGFB1, TGFB2, ADAMTS3, ASAMTS12, MMP1. Downregulated following CS exposure: E-CADHERIN, EGF, G-TYPE RECEPTOR, EPPK1, MAP7, BIK, MMP14, MUC1, EVA1, NRP3, FOS, TNSF10, MMP7, MUC20, TMPRSS3	
Vivarelli, F., et al., 2021	Cigarette	2s puff with 35ml volume and 30s intervals for 20 mins never for more than 3h/d. It was 4d/wk for 4 wks		Rats	Sprague Dawley rats	Upregulated following CS exposure: IL-13, IL-10, IL-12, TNF-alpha, INF-gamma, CSF2, CCL3, COX, CYP1A1, CYP2A1/2, CYP2B1/2, CYP2E1, JNK, ERK1/2, p38 MAPK.	Upregulated following CS exposure: 8-oxo-dG
Voinsky, I. and D. Gurwitz, 2020	Smoking	Smokers vs non-smokers	N/A	Human	Bronchial epithelial cells	Upregulated following CS exposure: TMPRSS4, NQO1, ALDH3A1, GPX2, AKR1C2, PIR, MUC5AC, ME1, CLDN10, CX3CL1, TXN, PPP1R16B, FAM114A1, SRPX2, S100A10, TRIM16, UGT1A1	

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Wang, G., et al., 2021	Cigarette, nicotine, BaP, NNK and pseudovirus	CSE(5-20%) for 0-72hrs; Nicotine 2.5-10umol/L for 0-72hrs; NNK at 5umol/L for 48hrs; BaP at 5umol/L for 48hrs; Mice exposed to CS via 750ug TPM/L for 20d, BaP at 100mg/kg twice a wk for 5 ks and NNK treatment at 50mg/kg twice a wk for 5 wks. Exposure with Pseudovirions involves incubation with 100uL of media for 48hrs.		Human and mice	16HBE cell line, BEAS2B cell line and lung samples (tissue adjacent to lung cancer); Mice A/J	Upregulated following CS exposure: ACE2, IL1ALPHA, IL-1BETA, IL-2, IL-6, TNFALPHA, SKP2. Downregulated following CS exposure: ACE2.	Upregulated following CS exposure: SKP2. Downregulated following CS exposure: ACE2.
Wang, Q., et al., 2014	CSE	1-5% CSE for up to 5d	13mg tar; 1.2mg nicotine per cigarette	Human	BEAS-2B cell line	Upregulated following CS exposure: uPAR, AKT.	Upregulated following CS exposure: N-cadherin, Vimentin, Alpha-SMA, uPAR, p-AKT. Downregulated following CS exposure: E-cadherin.
Wang, Q., et al., 2020	E-cig with or without nicotine	3.3s/puff, twice per minute with 70mL puff volume. Whole body exposure for 2hrs/d, 5d/wk for 30d with a 2.0L/min flowrate in the exposure chamber	Joytech VTC mini (SciReq, Montreal Canada)	Mice	C57BL/6J mice; nAChR α7 KO (α7 nicotinic acetylcholine receptor knockout mice); nAChR α7 CreCC10 mice (clara/club-cell-specific nAChR α7 deletion)	Upregulated following e-cig with nicotine: MMP8, TMP3. Downregulated following e-cig with nicotine: NECTIN1, SKIL, LDLR, COL1A2, COL4A1. Downregulated following e-cig: MMP8, MMP9, COL1A2, COL4A1.	Upregulated following e-cig with nicotine: p50, p105, ACE2, MMP9, MMP2, MMP12, PA1-1. Upregulated following e-cig exposure: MMP2. Downregulated following e-cig with nicotine: COL1A1. Downregulated following e-cig alone: ACE2, MMP8, MMP9, fibronectin.

S2 continued…

Reference	Exposure		Model			Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Wang, Y., et al., 2001	Nicotine	1uM nicotine for 3 days	N/A	Human	Bronchial epithelial cells and airway epithelial cells	Upregulated following nicotine exposure: CHRNA7	Upregulated following nicotine exposure: CHRNA7
Wark, P., et al., 2021	Cigarette	Pack years smoked	-	Human	Primary bronchial epithelial cells	No significant difference in any genes	
West, K., et al., 2003	Nicotine or NNK	10uM nicotine for 60mins; 1uM NNK added for 45mins	N/A	Human	Normal bronchial epithelial cells and small airway epithelial cells	Upregulated following CS exposure: CHRNA7. Upregulated following nicotine exposure: CHRNA3, CHRNA4, GSK-3, P70S6K, 4EBP1, FKHR.	Upregulated following CS exposure: AKT. Upregulated following nicotine exposure: P-AKT, P13K.
Wick, K. et al., 2022	E-cig	10puffs/min for 20-60min/d for 3d.	JUUL aerosol (Mint flavoured JUUL pod containing 0.7ml 5% nicotine salt e-liquid)	Human	Primary AT2 cells	Upregulated following e-cig with nicotine exposure: SLC7A11, SLC5A3, SLC38A2, CYP1A1, CYP1B1, AKR1C2, PGM2L1. Downregulated following e-cig with nicotine exposure: CCL2, CXCL2, EGR1, CFTR, JUN, ZNF385B, NOD1, SRSF5, FOS.	

S2 continued…

Reference	Exposure		Model			Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Wohnhaas, C., et al., 2021	Cigarette	Before differentiation CSE 4 cigarettes, 3/wk for 28d; after differentiation CSE was on four consecutive days	3R4F	Human	Small airway epithelial cells	Upregulation following CS exposure: CYP1A1, CYP1B1, ALDH1A3, MGST1, GPX2, AKR1C2, AKR1C1, AKR1C3, AKR1B10, TKT, ALDH3A1, ADH7, TALDO1, GCLC, TXN, GSTP1, PRDX1, NQO1, FTL, FTH1, MUC1, CXCL17, CXCL8, RARRES2, S100A8, S100A9, RNASE1, LCN2, BP1FA2, GPX2, GPX1, SAA1, SAA2, EGFR, TMPRSS4. Downregulation following CS exposure: CYP2F1	Upregulated following CS exposure: MUC5AC
Yang, J., et al., 2017	Smoking	Smokers vs non-smokers	N/A	Human	Proximal airway vs distal airway	Upregulated following CS exposure: UPK1B, GPX2, CYP26A1, CD44, ADH7, CYP4F3, IL1R2. Downregulated following CS exposure: LTF, SCGB3A2, SFPTB, MGP, WIF, TACR1, FOLR1, SOX9, TLR4, FOXA2, ARX, NPR3.	
Yang, J., et al., 2021	SARS-CoV-2 pseudovirus and cigarette	6h incubation at 37 degrees; CS	N/A	Human and mice	BEAS-2B cell line and MLE-12	Upregulated following CS exposure: ACE2. Downregulated following CS exposure: MIZ1	Upregulated following CS exposure: ACE2.
Yu, H., et al., 2011	Cigarette smoke	50mls one puff/min	N/A	Cell line	16HBE	Upregulated following CS exposure: NRG1BETA, ERBB3, MUC5AC	Upregulated following CS exposure: NRG1BETA, ERBB3, MUC5AC, P-ERK, JNK, TACE

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Yu, X., et al., 1992	Cigarette	No exposure vs acute cigarette smoke exposure for 5 mins or chronic cigarette smoke exposure 1hr/d for 30d.		Rats	Lungs	Upregulated following CS exposure: ACE.	
Zhang, L., et al., 2013	Cigarette smoke	8 x 35ml puffs for 2s/1min/cigarette, cell culture medium. Cell culture medium exposed to smoke for 6 continuous hrs	Kentucky 3R4F reference cigarette	Human	Primary human bronchial epithelial cells	Upregulated following CS exposure: RAC1, CDC42, P120CTN.	
Zhang, L., et al., 2013	Cigarette condensate	4h		Human	Primary Human bronchial epithelial cells		Upregulated following CS exposure: TyrP EGFR, pSrc, p-JNK. Downregulated following CS exposure: MUC1-CT, P120CTN, BETA-CTN, E-CADHERIN, p-AKT.
Zhang, L., et al., 2014	Cigarette smoke	cigarette condensate		Human	Primary human bronchial epithelial cells		Upregulated following CS exposure: MUC1-N. Downregulated following CS exposure: Cadherin, p120ctn.
Zhang, L., et al., 2016	Cigarette condensate	6 hours of cigarette burning		Human	Bronchial epithelial cells	Upregulated following CS exposure: Fos, Fra1, FosB	Upregulated following CS exposure: MUC1-CT, P120CTN, ERK-P

S2 continued…

Reference	Exposure			Model		Findings	
	Type	Dose and time	Brand	Species	Cell type	Gene (mRNA)	Protein
Zhang, Y., et al., 2021.	Cigarette	5 commercial cigarettes for two 30min sessions/d for 4 wks		Mice	C57 mice MLE-12 (AT2 cell line and AT2 cells	Upregulated following CS exposure: p21, p16, PARAP1. Downregulated following CS exposure: SIRT1, COXIV, LC3, LAMP1.	Upregulated following CS exposure: P16, P21, LC3.
Zhao, Y., et al., 2013	CSE	0-100ug/ml for 24h, 48h or 20wk	1R4F (9mg Tar; 0.8mg Nicotine per cigarette)	Human	HBE cell line	Upregulated following CS exposure: IL6.	Upregulated following CS exposure: N-cadherin, vimentin, p-p65, IL6, NF-KB. Downregulated following CS exposure: E-cadherin.
Zia, S., et al., 1997	Nicotine and cigarette smoke	0, 10uM, or 1mM of nicotine for 24hrs; Non-smoker and heavy smoker	N/A	Human and mice	Bronchial epithelial cells and mice lungs	Upregulated following CS exposure: CHRNA3. Upregulated following nicotine exposure: CHRNA7, CHRNA3, CHRNA5, CHRNA4.	Upregulated following CS exposure: Ca2+. Upregulated following nicotine exposure: CHRNA5.

* Studies declare funding from tobacco and/or e-cigarette manufacturers.

Abbreviations: CS = Cigarette smoke; E-cig = Electronic cigarette; h(rs) = hour(s); d = day; wk= week; CSC = Cigarette smoke condensate; CSE = Cigarette smoke extract.

Supplementary information 3: Quality scoring tool. The six domains included studies were scored against. Higher scores were assigned to studies investigating core viral-entry genes (ACE2 and TMPRSS2) as this is a key factor for addressing the aims of this study.

	Cell model		Route of exposure		Dosage	Gene and protein expression		Cytotoxicity		SARS-CoV-2 challenge
	In vitro	In vivo	In vitro	In vivo		Assays	Genes	In vitro	In vivo	
HIGH (3)	Primary human epithelial cells at ALI for 14+ days	NHP Animal Model OR tissues	Aerosolised exposure to cells	Intranasal exposure	Chosen dosage related to human exposure with appropriate controls or dose dependent curve with relevant controls	Studies followed up gene expression studies with multiple protein expression assays (knockout/ silencing/ inhibitor/ agonist)	Assessed the expression of more than two SARS-CoV-2 viral entry genes or different acetylcholine receptors	Use of multiple assays that measure cytotoxicity in cells	Assessed, oxidative stress and DNA damage and cell senescence markers/ infection. Confirmed with histology / BAL wash	WT SARS-CoV-2 infection
MEDIUM (2)	Primary human epithelial cells at ALI for less than 14 days OR Primary non-human epithelial cells at ALI OR unstated amount of time OR human epithelial cell lines for 14+ days	Hamster Animal Model OR tissues	Direct condensate application onto an ALI culture	Intratracheal	Chosen dosage not explained but appropriate controls or lack of human related dose	Protein (or immunostaining) and gene expression analysis performed	Assessed the expression of 1 or 2 of ACE2, TMPRSS2 or different acetylcholine receptors	Use of cell viability assay including oxidative stress markers OR DNA damage OR barrier integrity OR infected cells OR histology	Assessed oxidative stress markers OR DNA damage OR cell senescence confirmed with histology OR Infection	SARS-CoV-2 Pseudovirus infection OR SARS-CoV-2 infected samples
LOW (1)	Non-ALI cultures OR cell lines at ALI for less than 14 days OR non-differentiated microfluidics	Other Animal Model OR tissues	Direct condensate application into a submerged culture OR a biopsy sample from a smoker/ SARS-CoV-2 positive patient OR basal media in ALI/ microfluidics	Whole body exposure OR intraperitoneally OR unknown	Chosen dosage not explained with a lack of controls OR Chosen dosage related to human exposure with a lack of controls OR Smoker/non-smoker exposure biopsy	A protein or gene expression assay used	No SARS-CoV-2 or acetylcholine receptor genes of interest expression assessed	Did not assess cytotoxicity OR barrier integrity OR did not state methods	Damage not confirmed with histology or BAL	No infection

Abbreviations: ALI = Air-liquid interface; NHP = Non-human primates; BAL = Bronchoalveolar lavage; WT = Wild type.

Supplementary information 4: Quality assessment summary of all included studies. Where multiple scores were relevant for a single study, the highest applicable score is stated. Overall scores were classified by the average score. Values between: 1.00 and 1.25 were rated as VL = very low; 1.26 and 1.50 as L = Low; 1.51 and 2.00 as M = Medium; 2.01 and 3.00 as H = High.

Paper reference	Cell model		Route of exposure		Dosage	Gene and protein expression		Cytotoxicity		SARS-CoV-2 challenge	Overall score	
	In vitro	In vivo	In vitro	In vivo		Assays	Genes	In vitro	In vivo		Average	Rating
Agraval, H., et al., 2022	1		1		2	2	1	2		1	1.4	L
Akihiro Tsutsumi, 2020	2	1	1	3	3	3	1	2	2	1	1.9	M
Albano, G.D., et al., 2018	1		1		2	2	1	1		1	1.3	L
Aliee, H., et al., 2020	1		1		1	2	3	1		1	1.4	L
Al-Wadei, H.A.N., et al., 2010	1		1		3	3	2	1		1	1.7	M
Andrault, P., et al., 2019	1		1		3	3	1	3		1	1.9	M
Arredondo, J., et al., 2006	1		1		1	3	3	2		1	1.7	M
Basil, M. et al., 2022.	1	1	1	1	2	2	1	1	1	1	1.2	VL
Blank, U., et al., 1997	1		1		2	2	1	1		1	1.3	L
Cai, G., et al., 2020	1		1		1	1	3	1		1	1.3	L
Carlier, F. et al., 2021	3		1		1	2	1	2		1	1.6	M
Carlisle, D.L., et al., 2004	1		1		3	3	3	1		1	1.9	M
Chakladar, J., et al., 2020	1		1		1	1	3	1		1	1.3	L
Chen, P., et al., 2021.	1		1		2	3	1	1		1	1.4	L
Chen, Q., et al., 2021		1		3	2	2	1	1	2	3	1.9	M
Chen, Q., et al., 2022	3		2		2	2	3	2		3	2.4	H
Christian Ginzkey, 2012	1		1		3	3	2	3		1	2.0	M
Chu, M., et al., 2005	1		1		2	3	2	1		1	1.6	M
Chung, S., et al., 2015	1	1	1	1	2	3	1	2	2	1	1.5	L

S4 continued…

Paper reference	Cell model		Route of exposure		Dosage	Gene and protein expression		Cytotoxicity		SARS-CoV-2 challenge	Overall score	
	In vitro	In vivo	In vitro	In vivo		Assays	Genes	In vitro	In vivo		Average	Rating
Chung, S., et al., 2019	3	1	3	3	2	3	1	1	1	1	1.9	M
Cortijo, J., et al., 2011	3		1		2	3	1	2		1	1.9	M
Daniell, H., et al., 2022	1		1		1	2	2	1		3	1.6	M
Di Vincenzo, S. et al., 2022	3		3		1	2	1	2		1	1.9	M
Downs, C., et al., 2011	1		1		3	2	1	3		1	1.7	M
Du, H., et al., 2012	1		3		3	2	1	3		1	2.0	M
Ebrahimpour, A., et al., 2019	1		1		2	1	2	1		1	1.3	L
Eurlings, I., et al., 2014	1	1	1	1	3	3	1	3	2	1	1.7	M
Foronjy, R., et al., 2016	3	1	3	1	3	3	1	2	3	1	2.1	H
Fu, X., et al., 2009	1		1		3	3	3	1		1	1.9	M
Gahring, L.C., et al., 2017		1		1	2	3	1		2	1	1.6	M
Gao, H., et al., 2020	1		1		3	2	1	2		1	1.6	M
Gebel, S., et al., 2006		1		3	3	3	1		2	1	2.0	M
Geraghty, P., et al., 2014.	1	1	1	1	2	3	1	2	2	1	1.5	L
Ghosh, A., et al., 2021	2		3		2	2	3	2		2	2.3	H
Golovatch, P., et al., 2009		1		1	2	2	1		2	1	1.4	L
Gundavarapu, S., et al., 2012	2		2		3	3	3	2		1	2.3	H
Guo, J., et al., 2005	1		1		1	3	1	2		1	1.4	L
Haslbauer, J., et al., 2022	1		1		1	3	3	1		3	1.9	M
Hirschi-Budge, K. et al., 2022		1		3	2	3	1		1	1	1.7	M
Ho, Y., et al., 2005	1	1	1	1	3	3	3	2	2	1	1.8	M

S4 continued…

Paper reference	Cell model		Route of exposure		Dosage	Gene and protein expression		Cytotoxicity		SARS-CoV-2 challenge	Overall score	
	In vitro	In vivo	In vitro	In vivo		Assays	Genes	In vitro	In vivo		Average	Rating
Hou, W., et al., 2020	2		2		3	3	1	2		1	2.0	M
Hudlikar, R., et al., 2022.		1		1	1	3	1		2	1	1.4	L
Hung, Y., et al., 2016		1		1	2	3	2		3	1	1.9	M
Irie, H., et al., 2022		1		3	1	3	1		3	1	1.9	M
Izzotti, A., et al., 2010		1		1	1	2	1		1	1	1.1	VL
Jiang, J.-X., 2017	1	1	1	1	3	3	1	1	1	1	1.4	L
Kasper, B., et al., 2022	3		1		1	2	3	1		1	1.7	M
Lallai, V., Manca, L. and Fowler, C., 2021		1		1	2	2	3		1	1	1.6	M
Lam, D., et al., 2016	1		1		3	2	3	2		1	1.9	M
Latha, M., et al., 1991		1		1	1	1	1		1	1	1.0	VL
Lee, A., et al., 2020	1		1		1	1	2	1		1	1.1	VL
Lee, H. and J. Kim, 2013	1		1		3	1	1	2		1	1.4	L
Lee, I., et al., 2020	1	1	1	1	1	2	3	1	1	2	1.4	L
Lemjabbar, H., et al., 2003	1	1	1	3	2	3	1	2	2	1	1.7	M
Leung, J., et al., 2020	1		1		1	2	3	1		1	1.4	L
Li, D., et al., 2022		1		1	2	3	2		2	2	1.9	M
Li, E., et al., 2014	1		1		3	3	1	2		1	1.7	M
Li, F., et al., 2012		1		1	3	3	1		2	1	1.7	M
Li, F., et al., 2021	1		1		2	3	2	3		2	2.0	H
Li, G., et al., 2020	1		1		1	1	2	1		1	1.1	VL
Li, H., et al., 2021	3		2		2	3	1	3		1	2.1	H

S4 continued…

Paper reference	Cell model		Route of exposure		Dosage	Gene and protein expression		Cytotoxicity		SARS-CoV-2 challenge	Overall score	
	In vitro	In vivo	In vitro	In vivo		Assays	Genes	In vitro	In vivo		Average	Rating
Li, Q., et al., 2010	1		1		3	3	1	2		1	1.7	M
Li, Q., et al., 2011	1		1		2	3	3	1		1	1.7	M
Li, Q., et al., 2012	1		1		2	3	3	2		1	1.9	M
Li, W., et al., 2021	1		1		1	2	2	1		1	1.3	L
Lin, C., et al., 2021	1		1		1	2	3	2		1	1.6	M
Liu, A., et al., 2021	1	1	1	1	2	3	2	3	3	1	1.8	M
Lupacchini, L., et al., 2020	1		1		2	3	3	3		1	2.0	M
Lv, J., et al., 2021	1	3	1	2	1	3	2	1	2	3	1.9	M
Ma, L., et al., 2020	1	1	1	1	2	3	1	1	1	1	1.3	L
Maehira, F., et al., 1999		1		1	2	2	1		1	1	1.3	L
Mao, Y. and Feng, H., 2022	1		1		3	3	1	2		1	1.7	M
Marshall, K., et al., 2020		1		1	3	2	1		2	1	1.6	M
Martinez-Garcia, E., et al., 2008	1		1		3	3	3	1		1	1.9	M
Masso-Silva, J., et al., 2021		1		1	3	1	2		1	1	1.4	L
McAlinden, K., et al., 2021	1		1		3	2	2	3		1	1.9	M
Mebratu, Y., et al., 2011	2	1	1	1	3	3	1	2	2	1	1.7	M
Mishra, R., et al., 2016	2	1	2	1	3	3	1	2	1	1	1.7	M
Monzon, M., et al., 2011.	3		1		1	3	1	2		1	1.7	M
Nakayama, T., et al., 2021	3		2		3	2	3	3		3	2.7	H
Nath, S., et al., 2019		1		1	2	3	1		1	1	1.4	L
O'Donnell, R. et al., 2004	1		1		1	2	1	1		1	1.1	VL

S4 continued…

Paper reference	Cell model		Route of exposure		Dosage	Gene and protein expression		Cytotoxicity		SARS-CoV-2 challenge	Overall score	
	In vitro	In vivo	In vitro	In vivo		Assays	Genes	In vitro	In vivo		Average	Rating
Ogawa, F., et al., 2019	3		2		1	3	1	1		1	1.7	M
Osborne, J. et al., 2014	1		1		3	3	3	1		1	1.9	M
Peng, Y., et al., 2022	1		1		1	1	3	1		1	1.3	L
Phandthong, R., et al., 2022	1		3		3	2	2	2		2	2.1	H
Podowski, M., e al., 2012		1		1	1	3	1		2	1	1.4	L
Polk, W., 2012	1		1		3	3	1	3		1	1.9	M
Polosukhin, V., et al., 2011	2		1		1	3	1	2		1	1.6	M
Profita, M., et al., 2011	1		1		2	3	1	1		1	1.4	L
Qiao, Y., et al., 2021	1		1		1	3	2	2		3	1.9	M
Qixin Wang, 2020		1		1	2	3	3		2	1	1.9	M
Rangasamy, T., et al., 2009		1		1	2	3	1		2	1	1.6	M
Rayner, R., et al., 2022	3		2		3	1	1	2		1	1.9	M
Russo, P., et al., 2020	1		1		2	2	2	1		1	1.4	L
Sarkar, P. and Hayes, B., 2007	1		1		3	3	1	1		1	1.6	M
Shaykhiev, R., et al., 2013	3		2		2	2	1	2		1	1.9	M
Shen, H., et al., 2022	1	1	1	1	2	3	1	1	3	1	1.5	M
Sompel, K., et al., 2022	1	1	1	1	2	3	1	1	1	1	1.3	L
Staudt, M., et al., 2018	1		1		1	1	1	1		1	1.0	VL
Tan, L., et al., 2018	1	1	1	1	2	3	1	1	1	1	1.3	L
Tang, X., et al., 2021	1	1	1	1	3	3	1	3	3	1	1.8	M
Tomchaney, M., et al., 2020	2	1	3	1	2	2	2	2	1	3	1.9	M

S4 continued…

Paper reference	Cell model		Route of exposure		Dosage	Gene and protein expression		Cytotoxicity		SARS-CoV-2 challenge	Overall score	
	In vitro	In vivo	In vitro	In vivo		Assays	Genes	In vitro	In vivo		Average	Rating
Van der Toorn, M., et al., 2017	1		1		3	3	1	2		1	1.7	M
Veljkovic, E., et al., 2011	1		1		2	3	2	2		1	1.7	M
Vivarelli, F., et al., 2021		1		1	2	2	1		3	1	1.6	M
Voinsky, I. and D. Gurwitz, 2020	1		1		1	1	3	1		1	1.3	L
Wang, G., et al., 2021	1	1	1	1	3	3	3	1	2	2	1.8	M
Wang, Q., et al., 2014	1		1		2	3	1	2		1	1.6	M
Wang, Y., et al., 2001	1		1		1	3	2	1		1	1.4	L
Wark, P., et al., 2021	2		1		1	2	3	1		1	1.6	M
West, K., et al., 2003	1		1		3	3	3	3		1	2.1	H
Wick, K. et al., 2022	2		3		2	2	1	3		1	2.0	H
Wohnhaas, C., et al., 2021	3		3		3	2	3	2		1	2.4	H
Yang, J., et al., 2017	3		1		1	3	1	1		1	1.6	M
Yang, J., et al., 2021	1	1	1		1	3	2	1		2	1.5	L
Yu, H., et al., 2011	1		1		3	3	1	3		1	1.9	M
Yue, X., et al., 1992		1		1	1	1	2		1	1	1.1	VL
Zhang, L., et al., 2013	3		2		3	3	1	1		1	2.0	H
Zhang, L., et al., 2013	3		2		2	3	1	1		1	1.9	M
Zhang, L., et al., 2014	2		1		1	3	1	1		1	1.4	L
Zhang, L., et al., 2016	1		1		1	3	1	1		1	1.3	L
Zhang, Y., et al., 2021.	1	1	1	2	3	3	1	3	3	1	1.9	M
Zhao, Y., et al., 2013	1		1		3	3	1	3		1	1.9	M

S4 continued…

Paper reference	Cell model		Route of exposure		Dosage	Gene and protein expression		Cytotoxicity		SARS-CoV-2 challenge	Overall score	
	In vitro	In vivo	In vitro	In vivo		Assays	Genes	In vitro	In vivo		Average	Rating
Zia, S., et al., 1997	1	1	1	1	2	2	3	2	2	1	1.6	M

Supplementary information 5: Confidence scoring of genes extracted from all included studies after converting non-human GOI to their human homologues. Confidence scores combined study frequency, (the number of studies investigating the gene of interest (GOI)), consistency (the overall level of change of the GOI across all studies) and quality (the overall quality score of the studies from which each GOI was extracted). The higher the positive confidence score, the more robust the evidence for upregulation and the greater the negative confidence score, the more robust the evidence for downregulation.

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
ABCB6	10058	1.5				1.5					
ACE	1636	0.5	1.5								
ACE2	59272	12.5	5		-1.5	2	6.5	1	4	6.5	
ACTA2	59	1.0	4								
ACTL6B	51412	1.5									
ADAM17	6868	1.0	0								
ADAM19	8728	1.5									
ADAM8	101	1.5				-1.5					
ADAMTS12	81792	1.5									
ADAMTS3	9508	1.5									
ADAMTSL4	54507	1.0	1								
ADH7	131	5.0									
ADRB2	154		1								
AGER	177	1.5	1.5								
AGT	183					-1					
AGTR1	185	1.5									-1.5
AHR	196	1.5	1.5				1.5			1.5	
AIMP1	9255							1			
AJUBA	84962					0.5					
AKAP13	11214					1.5					
AKR1B10	57016	2.0									
AKR1C1	1645	3.5									
AKR1C2	1646	4.5				2					
AKR1C3	8644	2.0									
AKR1D1	6718	1.0									
AKT1	207	2.5	3.5					2	3		-2
ALDH1A3	220	2.0									
ALDH3A1	218	6.5	2								
AMOTL2	51421					0.5					
APOE	348	-1.5									
AR	367	1.0									
ARF6	382			1.5							
ARNT	405	1.5									
ARX	170302	-1.5									
ATXN1	6310	-1									
AURKA	6790		-1								
AVPR1A	552	-1.5									

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
BATF3	55509			0.5							
BAX	581	1.0									2
BCL2	596	2.5	1.5								-2
BCL2L1	598	1.5	1.5			1.5	1.5				
BEST3	144453					-0.5					
BIK	638	-3									
BIRC5	332	2		-1.5							
BMAL1	406	-1.5									
BPIFA2	140683	2.0									
BSG	682	1.5	2.5								
BTG2	7832					-1.5					
C3	718	2.0									
C4A	720			-1.5							
CA9	768	3.0									
CALML5	51806			-0.5							
CASP3	836	1.0	-0.5								
CASP7	840					-1.5					
CCL11	6356									1.5	
CCL2	6347	1.0	1.5			-2				1.5	
CCL20	6364	-1.5									
CCL3	6348	1.5									
CCL5	6352	-1.5									
CCL7	6354									1.5	
CCN2	1490		1								
CCND1	595	1.5	6.5						2.5		
CCNJ	54619	-1.5									
CCR2	729230		1.5			-1					
CCR7	1236			1.5							
CD151	977					1.5					
CD177	57126					-1					
CD40	958					-1					
CD44	960	3.0									
CD68	968		1								
CD74	972	1.5									
CDC42	998	2.0									
CDH1	999	-17.5	-16			-1.5	-1.5	-1.5			
CDH2	1000	1.5	3								
CDK9	1025	-1									
CDKN1A	1026	1.5	3								
CDKN2A	1029	1.5	1.5								
CDKN2C	1031		-1								
CEACAM5	1048	1.5									
CELSR1	9620	-1.5									

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
CES3	23491	1.0									
CFB	629	1.5									
CFTR	1080					-2					
CHAT	1103	2.0	3								
CHIT1	1118										
CHRM2	1129	2.0	2								
CHRM3	1131	2.5	2.5								
CHRNA1	1134	1.5	1.5					1.5	1.5		
CHRNA3	1136	1.5						5			
CHRNA4	1137							3.5			
CHRNA5	1138	1.5	1.5			-1.5		6	3		
CHRNA6	8973	-1.5						1.5			
CHRNA7	1139	1.5	1.5					10.5	4.5		
CHRNB2	1141	-1.5	1.5					1.5			
CHRNB4	1143	-1.5						1.5			
CHRNG	1146	-1.5	1.5								
CLCA1	1179	-1.5									
CLDN1	9076	-2		1.5							
CLDN10	9071	1.0									
CLDN3	1365	-1.5									
CLDN7	1366	2.0									
CLDN8	9073	2.0									
CLEC4M	10332	1.5	1.5								
CNN2	1265					-1.5					
COL12A1	1303					1.5					
COL1A1	1277	1.5	0.5				-1.5				
COL1A2	1278			-1.5		-1.5					
COL4A1	1282			-1.5		-1.5					
COX2	4513	5.0									
COX4I1	1327	-1.5									
CPT1A	1374		1.5								
CRIP1	1396			1.5		1.5					
CSF2	1437	3.0									
CSF3	1440	1.5									
CST6	1474					0.5					
CTNNB1	1499	-0.5	-2								
CTNND1	1500	2.0	-1.5								
CTSB	1508	1.5									
CTSD	1509	2.0									
CTSE	1510	1.0									
CTSG	1511	1.0									
CTSK	1513	2.5	1								
CTSL	1514	-1									

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
CTSS	1520	2.5	2.5	1.5							
CX3CL1	6376	1.0									
CXCL1	2919	2.0	1.5								
CXCL10	3627	-3								1.5	
CXCL17	284340	2.0									
CXCL2	2920					-2					
CXCL5	6374	0.5									
CXCL8	3576	12.5	3.5					1.5	1.5		2
CYBC1	79415		2								
CYGB	114757	-1.5									
CYP1A1	1543	13.0	1.5			2	1.5				
CYP1B1	1545	6.5				3.5					
CYP26A1	1592	1.5									
CYP2A13	1553	1.5	1.5				1.5				
CYP2A6	1548	1.5	1.5				1.5				
CYP2A7	1549	1.5									
CYP2B6	1555	1.5									
CYP2C19	1557	2.0									
CYP2C9	1559	2.0									
CYP2E1	1571	1.5									
CYP2F1	1572	-3.5									
CYP2J2	1573	1.0									
CYP3A4	1576	1.0				1.5					
CYP3A43	64816	1.0									
CYP3A5	1577	1.0									
CYP4F3	4051	1.5									
DBP	1628	1.5									
DEFB4B	1E+08	-1.5									
DLGAP5	9787			-0.5							
DMBT1	1755	2.0									
DNAI1	27019	-3									
DNASE1L2	1775			-0.5							
EGF	1950	0.0	1.5								
EGFR	1956	2.0	6.5						7		
EGR1	1958					-2					
EGR3	1960	1.0									
EIF4EBP1	1978							2			
ELK1	2002	-0.5									
ELN	2006		-1								
EN1	2019	-1.5									
EPPK1	83481	-1.5									
ERBB2	2064	-1.5	3								
ERBB3	2065	1.5	5								

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
ERBB4	2066	1.5									
ERCC6	2074	1.5									
ERN1	2081		1.5								
EVA1A	84141	-1.5									
FAM114A1	92689	1.0									
FCGRT	2217					-1					
FGFR3	2261	1.0	3								
FKBP8	23770	2.0									
FMO3	2328	2.0									
FN1	2335	1.5	3		-1.5				2		
FOLR1	2348	-1.5									
FOS	2352	-1.0				-2					
FOSB	2354	1.0									
FOSL1	8061	1.0									
FOXA2	3170	-1.5									
FOXA3	3171	1.5									
FOXJ1	2302	-3									
FOXO1	2308							2			
FRAT1	10023	-1									
FTH1	2495	2.0	-1.5								
FTL	2512	3.5									
FURIN	5045	2.0									
FZD9	8326	-1									
GATA3	2625	1.5									
GATA6	2627			1.5							
GCLC	2729	3.5									
GCLM	2730	1.5									
GEM	2669					-1.5					
GPX1	2876	3.5									
GPX2	2877	8.0									
GPX4	2879		-1.5								
GRHL3	57822			0.5							
GSK3B	2932							2			
GSS	2937		-1.5								
GSTP1	2950	2.0									
GUSB	2990	0.5									
H2AX	3014		3								
HDC	3067			-1.5							
HERPUD1	9709	1.5	1.5								
HES1	3280	-1.5									
HIF1A	3091	4.5	1.5								
HLA-DMA	3108		1.5								
HLA-DQA1	3117			1.5							

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
HLA-E	3133					-1.5					
HLA-F	3134					-1.5					
HLA-G	3135					-1.5					
HMGCS2	3158	-1.5									
HRAS	3265	3.0	3						2.5		
HRG	3273	1.0									
HSP90AA1	3320	1.5									
HSPA5	3309	1.5	3								
ICOSLG	23308			1.5							
IFNB1	3456		2								
IFNG	3458	1.5				-1				1.5	
IFNL3	282617									1.5	
IL10	3586	1.5								0.5	
IL12A	3592	1.5									
IL13	3596	1.5									
IL17A	3605									1.5	
IL17B	27190							1			
IL17RA	23765	1.0									
IL1A	3552	3				-1				2	
IL1B	3553	4.5						1			
IL1R2	7850	1.5		-1.5		-1.5					
IL2	3558	1.5				-1				1.5	
IL21	59067							1			
IL22	50616									1.5	
IL24	11009		1.5								
IL2RB	3560							1			
IL36A	27179	1.5									
IL5	3567									1.5	
IL6	3569	13.5	7			-1		1.5	1.5	2	2
ISG20	3669			0.5							
ITGAM	3684		1								
ITGAV	3685	-1									
IV	8114		1.5								
IVL	3713	1.5									
JAG1	182	-1.5									
JAK1	3716					-1					
JPH2	57158	1.0									
JUN	3725					-2					
KAT5	10524	1.5									
KCNK15	60598			-0.5							
KLHL13	90293	-1.5									
KRAS	3845	4.0	1.5								
KRT13	3860	1.5									

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
KRT14	3861	1.5									
KRT18	3875	-1.5									
KRT5	3852	-1.5									
KRT6A	3853	1.5									
KRT7	3855	-1.5									
KRT83	3889					1					
KRT9	3857	-1.5									
LAMP1	3916	-1.5									
LAMP3	27074	0.5									
LATS2	26524					0.5					
LCN2	3934	2.0									
LDLR	3949					-1.5					
LGALS7B	653499			0.5							
LMNA	4000		-1.5								
LTB4R	1241	1.0	1								
LTB4R2	56413			0.5							
LTF	4057	-1.5									
LY6G6D	58530			-1.5							
LY6H	4062	1.5									
MAGEA6	4105	1.5				1.5					
MAP1B	4131	1.5									
MAP1LC3A	84557	0									
MAP1LC3B	81631										2
MAP2K2	5605		1								
MAP2K3	5606					1.5					
MAP2K6	5608	1.5									
MAP3K1	4214	1.5									
MAP3K4	4216		1.5								
MAP7	9053	-1.5									
MAPK1	5594	7.5	20.5			-2		1			
MAPK14	1432	5.0	6					1.5	2		
MAPK3	5595	1.5						1			
MAPK8	5599	1.5	8								
MAPK9	5601	1.0	1								
MARCKSL1	65108			-1.5							
ME1	4199	1.0									
MGMT	4255	-2									
MGP	4256	-1.5									
MGST1	4257	2.0									
MKI67	4288								2		
MMP1	4312	3.0									
MMP12	4321	3.0					1.5				
MMP14	4323	-1.5									

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
MMP2	4313	1.5	0		1.5		1.5				
MMP3	4314	1.5									
MMP7	4316	-1.5									
MMP8	4317			-1.5	-1.5	1.5					
MMP9	4318	4.0	0.5	-1.5	-1.5		1.5				
MPHOSPH8	54737	-1									
MS4A1	931			1.5							
MSS51	118490	1.5									
MT1A	4489	2.0									
MT1X	4501	2.0				-0.5					
MT2A	4502					-0.5					
MTOR	2475		1.5								-2
MUC1	4582	0.5	0.5								
MUC20	200958	-1.5									
MUC5AC	4586	10.0	8.5					2	2		
MUC5B	727897	-1.5	1.5								
MUCL1	118430	1.5									
MYB	4602	-1.5									
MYBL1	4603	2.0									
MYC	4609		2								
MYD88	4615	1.5	1.5								
MYL5	4636					-0.5					
NCAM1	4684							1.5			
NECTIN1	5818					-1.5					
NEFM	4741							1.5			
NEURL3	93082					-0.5					
NEUROD1	4760							1.5	1.5		
NFE2	4778		-3								
NFE2L2	4780	0.0	1.5				1.5				
NFKB1	4790	5.5	8.5			-1	3	1			
NFKBIA	4792		6								
NFKBIB	4793					-1					
NKRF	55922	1.0									
NLRP3	114548	-1.5									
NOD1	10392					-2					
NOS2	4843	1.5									
NOTCH1	4851	-1.5									
NOX4	50507		1								
NPAS2	4862	-1.5									
NPR3	4883	-1.5									
NQO1	1728	8.0	2								
NR1D1	9572	3.0				-0.5					
NR1D2	9975	2.0									

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
NR3C2	4306					-1					
NRARP	441478	-1									
NRG1	3064	1.5	1.5								
NRP1	8829	-1.5									
OCLN	1E+08	-3.5	-1.5								
OGG1	4968		2.5								
OSGIN1	29948	1.5									
PAK3	5063	1.5									
PARD3	56288	-1.5									
PARD6A	50855	-1.5									
PARG	8505	0.5									
PARP1	142	1.5									
PAX3	5077							1.5			
PCNA	5111	1.5									
PDK2	5164	-1									
PEBP1	5037		1								
PGM2L1	283209					2					
PIGR	5284	1.5									
PIK3CA	5290		1.5			-1			4.5		
PIR	8544	1.0									
PLAUR	5329	1.5	1.5								
PLXDC1	57125			-0.5							
PPARG	5468	-2									
PPP1R16B	26051	1.0									
PRDX1	5052	2.0									
PRKCA	5578	-1				1.5		1.5	1		
PRKCI	5584	0.0									
PRNP	5621	-1.5									
PSMB9	5698					-1.5					
PTEN	5728	-1.5									
PTPN11	5781	1.5	1.5								
RAC1	5879	3.0	1								
RAF1	5894	1.0	1.5								
RARRES2	5919	2.0									
RASL11A	387496	-1.5									
RASSF1	11186	-2									
RELA	5970	2.0	3						-1.5		
RETNLB	84666	-1.5									
RNASE1	6035	2.0									
RND3	390					0.5					
ROS1	6098	1.5							1		
RPS6	6194							1			
RPS6KB1	6198							2			

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
S100A10	6281	1.0									
S100A8	6279	2.0		-1.5							
S100A9	6280	2.0									
SAA1	6288	2.0									
SAA2	6289	2.0									
SCGB1A1	7356	-1.5	-1.5								
SCGB3A2	117156	-1									
SDR39U1	56948	-1									
SERPINB2	5055					0.5					
SERPINE1	5054	1.5					1.5				
SFN	2810	1.5									
SFTPB	6439	-1.5									
SGK1	6446					-0.5					
SGPP1	81537					1.5					
SIRT1	23411	-1.5									
SKIL	6498					-1.5					
SKP2	6502	1.5	1.5								
SLC38A1	81539	-1.5									
SLC38A2	54407					2					
SLC5A3	6526					2					
SLC5A8	160728			0.5							
SLC7A1	6541					-1					
SLC7A11	23657		-1.5			2					
SLC9A5	6553	1.5				1.5					
SMAD2	4087		1								
SMAD3	4088		1.5								
SMPD3	55512	1.0									
SNAI1	6615	1.5									
SNAI2	6591	4.5	1.5								
SNAI3	333929	-1.5									
SOD1	6647		1.5				1.5				
SOX14	8403	1.0									
SOX9	6662	-1.5									
SPDEF	25803	1.5									
SPP1	6696	-2									
SQSTM1	8878										-2
SRC	6714	3.0	3								
SRPX2	27286	1.0									
SRSF5	6430					-2					
ST6GALNAC3	256435	1.5									
STAB1	23166					1.5					
STAT1	6772	1.5	1.5			-1					

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
STAT3	6774		3								
STEAP4	79689	1.5									
TACR1	6869	-1.5									
TACSTD2	4070	2.0	2								
TADA2A	6871	-1									
TALDO1	6888	2.0									
TFRC	7037		1.5								
TGFA	7039	-1.5									
TGFB1	7040	1.0	4			-1					
TGFB2	7042	1.5									
TIMP1	7076	1.5									
TIMP2	7077	-1.5									
TJP1	7082	-1.5						-1.5			-2
TKT	7086	2.0									
TLR4	7099	-1.5									
TLR6	10333					1.5					
TLR9	54106	2.0	2			-1					
TMEM171	134285			-0.5							
TMOD1	7111	-1.5									
TMPRSS2	7113	4.5	1.5		-2		2		2		
TMPRSS3	64699	-1.5									
TMPRSS4	56649	3.0									
TNC	3371			-1.5							
TNF	7124	7.0	3			-1		1	0		2
TNFRSF19	55504	1.5									
TNFRSF1B	7133							1			
TNFRSF4	7293							1			
TNFSF10	8743	-1.5									
TP53	7157	-0.5							-1.5		
TP63	8626	-1.5									
TPM3	7170					1.5					
TRAP1	10131							1.5			
TRAPPC4	51399	-1									
TRIM16	10626	1.0									
TRPC7	57113	-1									
TWIST1	7291	1.5						1			
TWIST2	117581	1.5									
TXK	7294			0.5							
TXN	7295	3.0									
TXNDC16	57544			1.5							
TYRP1	7306		1.5								
UGT1A1	54658	1.0									
UGT2B15	7366	2.0									

S5 continued…

Human translated gene (NCBI)	Gene ID	Cigarette smoke exposure		E-cigarette exposure (No nicotine)		E-cigarette exposure (Nicotine)		Nicotine alone exposure		SARS-CoV-2 exposure	
		mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein
UGT2B17	7367	2.0									
UPK1B	7348	1.5									
VASP	7408					-1.5					
VEGFA	7422	3.0	1.5						4		
VIM	7431	9.5	8						2		
VNN1	8876	1.5									
WIF1	11197	-1.5									
XIST	7503	1.0									
XPO1	7514	-1.5	-1.5			-1.5	-1.5				
ZBTB17	7709	-1									
ZEB1	6935	1.5									
ZHX2	22882								1.5		
ZMYND11	10771	-2									
ZNF385B	151126					-2					

References:

1. Agraval H, Sharma JR, Prakash N, Yadav UCS. Fisetin suppresses cigarette smoke extract-induced epithelial to mesenchymal transition of airway epithelial cells through regulating COX-2/MMPs/13-catenin pathway. Chemico-Biological Interactions. 2022;351.
2. Al-Wadei HAN, Al-Wadei MH, Masi T, Schuller HM. Chronic exposure to estrogen and the tobacco carcinogen NNK cooperatively modulates nicotinic receptors in small airway epithelial cells. Lung Cancer. 2010;69(1):33-9.
3. Albano GD, Bonanno A, Moscato M, Anzalone G, Di Sano C, Riccobono L, et al. Crosstalk between mAChRM3 and beta 2AR, via acetylcholine PI3/PKC/PBEP1/Raf-1 MEK1/2/ERK1/2 pathway activation, in human bronchial epithelial cells after long-term cigarette smoke exposure. Life Sciences. 2018;192:99-109.
4. Aliee H, Massip F, Qi C, Stella de Biase M, van Nijnatten JL, Kersten ETG, et al. Determinants of SARS-CoV-2 receptor gene expression in upper and lower airways. medRxiv : the preprint server for health sciences. 2020.
5. Andrault P-M, Schamberger AC, Chazeirat T, Sizaret D, Renault J, Staab-Weijnitz CA, et al. Cigarette smoke induces overexpression of active human cathepsin S in lungs from current smokers with or without COPD. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2019;317(5):L625-L38.
6. Arredondo J, Chernyavsky AI, Grando SA. The nicotinic receptor antagonists abolish pathobiologic effects of tobacco-derived nitrosamines on BEP2D cells. Journal of Cancer Research and Clinical Oncology. 2006;132(10):653-63.
7. Basil MC, Cardenas-Diaz FL, Kathiriya JJ, Morley MP, Carl J, Brumwell AN, et al. Human distal airways contain a multipotent secretory cell that can regenerate alveoli. Nature. 2022;604(7904):120-+.
8. Blank U, Ruckes C, Clauss W, Weber WM. Effects of nicotine on human nasal epithelium: evidence for nicotinic receptors in non-excitable cells. Pflugers Archiv - European Journal of Physiology. 1997;434(5):581-6.
9. Cai G, Bosse Y, Xiao F, Kheradmand F, Amos CI. Tobacco Smoking Increases the Lung Gene Expression of ACE2, the Receptor of SARS-CoV-2. American Journal of Respiratory and Critical Care Medicine. 2020;201(12):1557-9.
10. Carlier FM, Detry B, Lecocq M, Collin AM, Plante-Bordeneuve T, Verleden SE, et al. The memory of airway epithelium damage in smokers and COPD patients. bioRxiv. 2021;22.
11. Carlisle DL, Hopkins TM, Gaither-Davis A, Silhanek MJ, Luketich JD, Christie NA, et al. Nicotine signals through muscle-type and neuronal nicotinic acetylcholine receptors in both human bronchial epithelial cells and airway fibroblasts. Respiratory Research. 2004;5(27-28).
12. Chakladar J, Shende N, Li WT, Rajasekaran M, Chang EY, Ongkeko WM. Smoking-Mediated Upregulation of the Androgen Pathway Leads to Increased SARS-CoV-2 Susceptibility. International Journal of Molecular Sciences. 2020;21(10).
13. Chen P, Jiang P, Chen J, Yang Y, Guo X. XIST promotes apoptosis and the inflammatory response in CSE-stimulated cells via the miR-200c-3p/EGR3 axis. Bmc Pulmonary Medicine. 2021;21(1).
14. Chen Q, Huang X-Y, Tian Y, Fan C, Sun M, Zhou C, et al. The Infection and Pathogenicity of SARS-CoV-2 Variant B.1.351 in hACE2 Mice. Virologica Sinica. 2021;36(5):1232-5.
15. Chen Q, Langenbach S, Li M, Xia YC, Gao X, Gartner MJ, et al. ACE2 Expression in Organotypic Human Airway Epithelial Cultures and Airway Biopsies. Frontiers in Pharmacology. 2022;13.
16. Chu M, Guo JJ, Chen CY. Long-term exposure to nicotine, via Ras pathway, induces cyclin D1 to stimulate G(1) cell cycle transition. Journal of Biological Chemistry. 2005;280(8):6369-79.
17. Chung S, Baumlin N, Dennis JS, Moore R, Salathe SF, Whitney PL, et al. Electronic Cigarette Vapor with Nicotine Causes Airway Mucociliary Dysfunction Preferentially via TRPA1 Receptors. American Journal of Respiratory and Critical Care Medicine. 2019;200(9):1134-45.
18. Chung S, Vu S, Filosto S, Goldkorn T. Src Regulates Cigarette Smoke-Induced Ceramide Generation via Neutral Sphingomyelinase 2 in the Airway Epithelium. American Journal of Respiratory Cell and Molecular Biology. 2015;52(6):738-48.
19. Cortijo J, Mata M, Milara J, Donet E, Gavalda A, Miralpeix M, et al. Aclidinium inhibits cholinergic and tobacco smoke-induced MUC5AC in human airways. European Respiratory Journal. 2011;37(2):244-54.
20. Daniell H, Nair SK, Shi Y, Wang P, Montone KT, Shaw PA, et al. Decrease in Angiotensin-Converting Enzyme activity but not concentration in plasma/lungs in COVID-19 patients offers clues for diagnosis/treatment. Molecular Therapy-Methods & Clinical Development. 2022;26:266-78.
21. Di Vincenzo S, Ninaber DK, Cipollina C, Ferraro M, Hiemstra PS, Pace E. Cigarette Smoke Impairs Airway Epithelial Wound Repair: Role of Modulation of Epithelial-Mesenchymal Transition Processes and Notch-1 Signaling. Antioxidants (Basel, Switzerland). 2022;11(10).

22. Downs CA, Montgomery DW, Merkle CJ. Age-related differences in cigarette smoke extract-induced H2O2 production by lung endothelial cells. Microvascular Research. 2011;82(3):311-7.
23. Du H, Sun J, Chen Z, Nie J, Tong J, Li J. CIGARETTE SMOKE-INDUCED FAILURE OF APOPTOSIS RESULTING IN ENHANCED NEOPLASTIC TRANSFORMATION IN HUMAN BRONCHIAL EPITHELIAL CELLS. Journal of Toxicology and Environmental Health-Part a-Current Issues. 2012;75(12):707-20.
24. Ebrahimpour A, Shrestha S, Bonnen MD, Eissa NT, Raghu G, Ghebre YT. Nicotine Modulates Growth Factors and MicroRNA to Promote Inflammatory and Fibrotic Processes. Journal of Pharmacology and Experimental Therapeutics. 2019;368(2):169-78.
25. Eurlings IMJ, Reynaert NL, van den Beucken T, Gosker HR, de Theije CC, Verhamme FM, et al. Cigarette Smoke Extract Induces a Phenotypic Shift in Epithelial Cells; Involvement of HIF1 alpha in Mesenchymal Transition. Plos One. 2014;9(10).
26. Foronjy RF, Salathe MA, Dabo AJ, Baumlin N, Cummins N, Eden E, et al. TLR9 expression is required for the development of cigarette smoke-induced emphysema in mice. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2016;311(1):L154-L66.
27. Fu XW, Lindstrom J, Spindel ER. Nicotine Activates and Up-Regulates Nicotinic Acetylcholine Receptors in Bronchial Epithelial Cells. American Journal of Respiratory Cell and Molecular Biology. 2009;41(1):93-9.
28. Gahring LC, Myers EJ, Dunn DM, Weise RB, Rogers SW. Lung epithelial response to cigarette smoke and modulation by the nicotinic alpha 7 receptor. Plos One. 2017;12(11).
29. Gao H, Ye G, Lin Y, Chi Y, Dong S. Benzo a pyrene at human blood equivalent level induces human lung epithelial cell invasion and migration via aryl hydrocarbon receptor signaling. Journal of Applied Toxicology. 2020;40(8):1087-98.
30. Gebel S, Gerstmayer B, Kuhl P, Borlak J, Meurrens K, Mueller T. The kinetics of transcriptomic changes induced by cigarette smoke in rat lungs reveals a specific program of defense, inflammation, and circadian clock gene expression. Toxicological Sciences. 2006;93(2):422-31.
31. Geraghty P, Hardigan A, Foronjy RF. Cigarette Smoke Activates the Proto-oncogene c-Src to Promote Airway Inflammation and Lung Tissue Destruction. American Journal of Respiratory Cell and Molecular Biology. 2014;50(3):559-70.
32. Ghosh A, Girish V, Yuan ML, Coakley RD, Wrennall JA, Alexis NE, et al. Combustible and electronic cigarette exposures increase ACE2 activity and SARS-CoV-2 Spike binding. American Journal of Respiratory and Critical Care Medicine. 2022;205(1):129-33.
33. Ginzkey C, Stueber T, Friehs G, Koehler C, Hackenberg S, Richter E, et al. Analysis of nicotine-induced DNA damage in cells of the human respiratory tract. Toxicology Letters. 2012;208(1):23-9.
34. Golovatch P, Mercer BA, Lemaitre V, Wallace A, Foronjy RF, D'Armiento J. ROLE FOR CATHEPSIN K IN EMPHYSEMA IN SMOKE-EXPOSED GUINEA PIGS. Experimental Lung Research. 2009;35(8):631-45.
35. Gundavarapu S, Wilder JA, Mishra NC, Rir-sima-ah J, Langley RJ, Singh SP, et al. Role of nicotinic receptors and acetylcholine in mucous cell metaplasia, hyperplasia, and airway mucus formation in vitro and in vivo. Journal of Allergy and Clinical Immunology. 2012;130(3):770-+.
36. Guo JJ, Chu M, Abbeyquaye T, Chen CY. Persistent nicotine treatment potentiates amplification of the dihydrofolate reductase gene in rat lung epithelial cells as a consequence of Ras activation. Journal of Biological Chemistry. 2005;280(34):30422-31.
37. Haslbauer JD, Stalder A, Zinner C, Bassetti S, Mertz KD, Went P, et al. Immunohistochemical and Transcriptional Analysis of SARS-CoV-2 Entry Factors and Renin-Angiotensin-Aldosterone System Components in Lethal COVID-19. Pathobiology. 2022;89(3):166-77.
38. Hirschi-Budge KM, Tsai KYF, Curtis KL, Davis GS, Theurer BK, Kruyer AMM, et al. RAGE signaling during tobacco smoke-induced lung inflammation and potential therapeutic utility of SAGEs. Bmc Pulmonary Medicine. 2022;22(1).
39. Ho YS, Chen CH, Wang YJ, Pestell RG, Albanese C, Chen RJ, et al. Tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces cell proliferation in normal human bronchial epithelial cells through NF kappa B activation and cyclin D1 up-regulation. Toxicology and Applied Pharmacology. 2005;205(2):133-48.
40. Hou W, Hu S, Yong KT, Zhang J, Ma H. Cigarette smoke-induced malignant transformation via STAT3 signalling in pulmonary epithelial cells in a lung-on-a-chip model. Bio-Design and Manufacturing. 2020;3(4):383-95.
41. Hudlikar RR, Sargsyan D, Cheng D, Kuo H-CD, Wu R, Su X, et al. Tobacco carcinogen 4- methyl(nitroso)amino -1-(3-pyridinyl)-1-butanone (NNK) drives metabolic rewiring and epigenetic reprograming in A/J mice lung cancer model and prevention with diallyl sulphide (DAS). Carcinogenesis. 2022;43(2):140-9.

42. Hung Y-H, Hsieh W-Y, Hsieh J-S, Liu F-C, Tsai C-H, Lu L-C, et al. Alternative Roles of STAT3 and MAPK Signaling Pathways in the MMPs Activation and Progression of Lung Injury Induced by Cigarette Smoke Exposure in ACE2 Knockout Mice. International Journal of Biological Sciences. 2016;12(4):454-65.
43. Irie H, Ozaki M, Chubachi S, Hegab AE, Tsutsumi A, Kameyama N, et al. Short-term intermittent cigarette smoke exposure enhances alveolar type 2 cell stemness via fatty acid oxidation. Respiratory Research. 2022;23(1).
44. Izzotti A, Calin GA, Steele VE, Cartiglia C, Longobardi M, Croce CM, et al. Chemoprevention of Cigarette Smoke-Induced Alterations of MicroRNA Expression in Rat Lungs. Cancer Prevention Research. 2010;3(1):62-72.
45. Jiang J-X, Zhang S-J, Shen H-J, Guan Y, Liu Q, Zhao W, et al. Racl signaling regulates cigarette smoke-induced inflammation in the lung via the Erk1/2 MAPK and STAT3 pathways. Biochimica Et Biophysica Acta-Molecular Basis of Disease. 2017;1863(7):1778-88.
46. Kasper B, Yue X, Goldmann T, Guelsen A, Kugler C, Yu X, et al. Air exposure and cell differentiation are essential for investigation of SARS-CoV-2 entry genes in human primary airway epithelial cells in vitro. Frontiers in Medicine. 2022;9.
47. Lallai V, Manca L, Fowler CD. E-cigarette vape and lung ACE2 expression: Implications for coronavirus vulnerability. Environmental Toxicology and Pharmacology. 2021;86.
48. Lam DCL, Luo SY, Fu KH, Lui MMS, Chan KH, Wistuba II, et al. Nicotinic acetylcholine receptor expression in human airway correlates with lung function. American Journal of Physiology - Lung Cellular and Molecular Physiology. 2016;310(3):L232-L9.
49. Latha MS, Vijayammal PL, Kurup PA. CHANGES IN THE GLYCOSAMINOGLYCANS AND GLYCOPROTEINS IN THE TISSUES IN RATS EXPOSED TO CIGARETTE-SMOKE. Atherosclerosis. 1991;86(1):49-54.
50. Lee AC, Chakladar J, Li WT, Chen C, Chang EY, Wang-Rodriguez J, et al. Tobacco, but Not Nicotine and Flavor-Less Electronic Cigarettes, Induces ACE2 and Immune Dysregulation. International Journal of Molecular Sciences. 2020;21(15).
51. Lee HS, Kim J. Cigarette smoke inhibits nasal airway epithelial cell growth and survival. International Forum of Allergy & Rhinology. 2013;3(3):188-92.
52. Lee IT, Nakayama T, Wu C-T, Goltsev Y, Jiang S, Gall PA, et al. ACE2 localizes to the respiratory cilia and is not increased by ACE inhibitors or ARBs. Nature Communications. 2020;11(1).
53. Lemjabbar H, Li DZ, Gallup M, Sidhu S, Drori E, Basbaum C. Tobacco smoke-induced lung cell proliferation mediated by tumor necrosis factor alpha-converting enzyme and amphiregulin. Journal of Biological Chemistry. 2003;278(28):26202-7.
54. Leung JM, Yang CX, Sin DD. COVID-19 and nicotine as a mediator of ACE-2. European Respiratory Journal. 2020;55(6).
55. Li D, Xiong L, Pan G, Wang T, Li R, Zhu L, et al. Molecular imaging on ACE2-dependent transocular infection of coronavirus. Journal of Medical Virology. 2022;94(10):4878-89.
56. Li E, Xu Z, Liu F, Wang H, Wen J, Shao S, et al. Continual Exposure to Cigarette Smoke Extracts Induces Tumor-Like Transformation of Human Nontumor Bronchial Epithelial Cells in a Microfluidic Chip. Journal of Thoracic Oncology. 2014;9(8):1091-100.
57. Li F, Li J, Wang P-H, Yang N, Huang J, Ou J, et al. SARS-CoV-2 spike promotes inflammation and apoptosis through autophagy by ROS-suppressed PI3K/AKT/mTOR signaling. Biochimica Et Biophysica Acta-Molecular Basis of Disease. 2021;1867(12).
58. Li F-f, Shen J, Shen H-j, Zhang X, Cao R, Zhang Y, et al. Shp2 Plays an Important Role in Acute Cigarette Smoke-Mediated Lung Inflammation. Journal of Immunology. 2012;189(6):3159-67.
59. Li G, He X, Zhang L, Ran Q, Wang J, Xiong A, et al. Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19. Journal of Autoimmunity. 2020;112.
60. Li H, Cui L, Liu Q, Dou S, Wang W, Xie M, et al. Ginsenoside Rb3 Alleviates CSE-induced TROP2 Upregulation through p38 MAPK and NF-kappa B Pathways in Basal Cells. American Journal of Respiratory Cell and Molecular Biology. 2021;64(6):747-59.
61. Li Q, Kolosov VP, Perelman JM, Zhou XD. Src-JNK-dependent pathway in cigarette smoke-induced mucous hypersecretion in airway epithelial cells. Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology. 2010;4(4):363-73.
62. Li Q, Zhou X, Kolosov VP, Perelman JM. The Expression and Pharmacological Characterization of Nicotinic Acetylcholine Receptor Subunits in HBE16 Airway Epithelial Cells. Cell Biochemistry and Biophysics. 2012;62(3):421-31.

63. Li Q, Zhou XD, Kolosov VP, Perelman JM. Nicotine reduces TNF-alpha expression through a alpha7 nAChR/MyD88/NF-kappaB pathway in HBE16 airway epithelial cells. Cellular Physiology and Biochemistry. 2011;27(5):605-12.
64. Li W, Zheng R, Liang R, Wu B, Wang C, Zhuo L, et al. Effects of Smoking on ACE2 Expression Pattern: Risk and Severity of SARS-CoV-2 Infection. Clinical Laboratory. 2021;67(7):1633-40.
65. Lin C-R, Bahmed K, Simborio H, Hayek H, Bolla S, Marchetti N, et al. Expression of SARS-CoV-2 Entry Factors in Human Alveolar Type II Cells in Aging and Emphysema. Biomedicines. 2021;9(7).
66. Liu A, Zhang X, Li R, Zheng M, Yang S, Dai L, et al. Overexpression of the SARS-CoV-2 receptor ACE2 is induced by cigarette smoke in bronchial and alveolar epithelia. Journal of Pathology. 2021;253(1):17-30.
67. Lupacchini L, Maggi F, Tomino C, De Dominicis C, Mollinari C, Fini M, et al. Nicotine Changes Airway Epithelial Phenotype and May Increase the SARS-COV-2 Infection Severity. Molecules. 2020;26(1).
68. Lv J, Yu P, Wang Z, Deng W, Bao L, Liu J, et al. ACE2 expression is regulated by AhR in SARS-CoV-2-infected macaques. Cellular and Molecular Immunology. 2021;18(5):1308-10.
69. Ma L, Jiang M, Zhao X, Sun J, Pan Q, Chu S. Cigarette and IL-17A synergistically induce bronchial epithelial-mesenchymal transition via activating IL-17R/NF-kappa B signaling. Bmc Pulmonary Medicine. 2020;20(1).
70. Maehira F, Miyagi I, Asato T, Eguchi Y, Takei H, Nakatsuki K, et al. Alterations of protein kinase C, 8-hydroxydeoxyguanosine, and K-ras oncogene in rat lungs exposed to passive smoking. Clinica Chimica Acta. 1999;289(1-2):133-44.
71. Mao Y, Feng H. Vitamin D3 alleviates cigarette smoke extract-mediated epithelial-mesenchymal transition and fibrogenesis by upregulating CC16 expression in bronchial epithelial cells. Experimental and Therapeutic Medicine. 2022;23(5).
72. Marshall K, Liu Z, Olfert IM, Gao W. Chronic electronic cigarette use elicits molecular changes related to pulmonary pathogenesis. Toxicology and Applied Pharmacology. 2020;406.
73. Martinez-Garcia E, Irigoyen M, Anso E, Martinez-Irujo JJ, Rouzaut A. Recurrent exposure to nicotine differentiates human bronchial epithelial cells via epidermal growth factor receptor activation. Toxicology & Applied Pharmacology. 2008;228(3):334-42.
74. Masso-Silva JA, Moshensky A, Shin J, Olay J, Nilaad S, Advani I, et al. Chronic E-Cigarette Aerosol Inhalation Alters the Immune State of the Lungs and Increases ACE2 Expression, Raising Concern for Altered Response and Susceptibility to SARS-CoV-2. Frontiers in Physiology. 2021;12.
75. McAlinden KD, Lu W, Ferdowsi PV, Myers S, Markos J, Larby J, et al. Electronic Cigarette Aerosol Is Cytotoxic and Increases ACE2 Expression on Human Airway Epithelial Cells: Implications for SARS-CoV-2 (COVID-19). Journal of Clinical Medicine. 2021;10(5).
76. Mebratu YA, Schwalm K, Smith KR, Schuyler M, Tesfaigzi Y. Cigarette Smoke Suppresses Bik To Cause Epithelial Cell Hyperplasia and Mucous Cell Metaplasia. American Journal of Respiratory and Critical Care Medicine. 2011;183(11):1531-8.
77. Mishra R, Foster D, Vasu VT, Thaikoottathil JV, Kosmider B, Chu HW, et al. Cigarette Smoke Induces Human Epidermal Receptor 2-Dependent Changes in Epithelial Permeability. American Journal of Respiratory Cell and Molecular Biology. 2016;54(6):853-64.
78. Monzon ME, Forteza RM, Casalino-Matsuda SM. MCP-1/CCR2B-dependent loop upregulates MUC5AC and MUC5B in human airway epithelium. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2011;300(2):L204-L15.
79. Nakayama T, Lee IT, Jiang S, Matter MS, Yan CH, Overdevest JB, et al. Determinants of SARS-CoV-2 entry and replication in airway mucosal tissue and susceptibility in smokers. Cell Reports Medicine. 2021;2(10).
80. Nath S, Doherty DF, Foronjy RF, Ohlmeyer M, Poon J, Dabo AJ, et al. Protein Phosphatase 2A Prevents Cigarette Smoke-Induced Cathepsin S and Loss of Lung Function. American Journal of Respiratory and Critical Care Medicine. 2019;199.
81. O'Donnell RA, Richter A, Ward J, Angco G, Mehta A, Rousseau K, et al. Expression of ErbB receptors and mucins in the airways of long term current smokers. Thorax. 2004;59(12):1032-40.
82. Ogawa F, Walters MS, Shafquat A, O'Beirne SL, Kaner RJ, Mezey JG, et al. Role of KRAS in regulating normal human airway basal cell differentiation. Respiratory Research. 2019;20(1).
83. Osborne JK, Guerra ML, Gonzales JX, McMillan EA, Minna JD, Cobb MH. NeuroD1 mediates nicotine-induced migration and invasion via regulation of the nicotinic acetylcholine receptor subunits in a subset of neural and neuroendocrine carcinomas. Molecular Biology of the Cell. 2014;25(11):1782-92.

84. Peng Y, Zhao-Ni W, Chen SY, Xu AR, Fang ZF, Sun J, et al. Angiotensin-converting enzyme 2 in peripheral lung club cells modulates the susceptibility to SARS-CoV-2 in chronic obstructive pulmonary disease. American Journal of Physiology - Lung Cellular and Molecular Physiology. 2022;322(5):L712-L21.
85. Phandthong R, Wong M, Song A, Martinez T, Talbot P. New Insights into How JUUL Electronic Cigarette Aerosols and Aerosol Constituents Affect SARS-CoV-2 Infection of Human Bronchial Epithelial Cells. bioRxiv : the preprint server for biology. 2022.
86. Podowski M, Calvi C, Metzger S, Misono K, Poonyagariyagorn H, Lopez-Mercado A, et al. Angiotensin receptor blockade attenuates cigarette smoke-induced lung injury and rescues lung architecture in mice. Journal of Clinical Investigation. 2012;122(1):229-40.
87. Polk WW. FH535 Potentiation of Cigarette Smoke Condensate Cytotoxicity is Associated With Changes in beta-Catenin and EGR-1 Signaling. International Journal of Toxicology. 2012;31(4):380-9.
88. Polosukhin VV, Cates JM, Lawson WE, Milstone AP, Matafonov AG, Massion PP, et al. Hypoxia-inducible factor-1 signalling promotes goblet cell hyperplasia in airway epithelium. Journal of Pathology. 2011;224(2):203-11.
89. Profita M, Bonanno A, Montalbano AM, Ferraro M, Siena L, Bruno A, et al. Cigarette smoke extract activates human bronchial epithelial cells affecting non-neuronal cholinergic system signalling in vitro. Life Sciences. 2011;89(1-2):36-43.
90. Qiao Y, Wang X-M, Mannan R, Pitchiaya S, Zhang Y, Wotring JW, et al. Targeting transcriptional regulation of SARS-CoV-2 entry factors ACE2 and TMPRSS2. Proceedings of the National Academy of Sciences of the United States of America. 2021;118(1).
91. Rangasamy T, Misra V, Zhen L, Tankersley CG, Tuder RM, Biswal S. Cigarette smoke-induced emphysema in A/J mice is associated with pulmonary oxidative stress, apoptosis of lung cells, and global alterations in gene expression. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2009;296(6):L888-L900.
92. Rayner RE, Wellmerling J, Makena P, Zhao J, Prasad GL, Cormet-Boyaka E. Transcriptomic Response of Primary Human Bronchial Cells to Repeated Exposures of Cigarette and ENDS Preparations. Cell Biochemistry and Biophysics. 2022;80(1):217-28.
93. Russo P, Bonassi S, Giacconi R, Malavolta M, Tomino C, Maggi F. COVID-19 and smoking: Is nicotine the hidden link? European Respiratory Journal. 2020;55(6) (no pagination).
94. Sarkar P, Hayes BE. Induction of COX-2 by acrolein in rat lung epithelial cells. Molecular and Cellular Biochemistry. 2007;301(1-2):191-9.
95. Shaykhiev R, Zuo W-L, Chao I, Fukui T, Witover B, Brekman A, et al. EGF shifts human airway basal cell fate toward a smoking-associated airway epithelial phenotype. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(29):12102-7.
96. Shen H-j, Jiang J-x, Yao H-y, Zhou H-y, Xie Q-m, Dai H-b, et al. Glycopyrronium bromide regulates cigarette smoke-induced epithelial mesenchymal transition by mediating ACh production. Journal of Molecular Structure. 2022;1266.
97. Sompel K, Dwyer-Nield LD, Smith AJ, Elango AP, Vanderlinden LA, Kopf K, et al. Loss of Frizzled 9 in Lung Cells Alters Epithelial Phenotype and Promotes Premalignant Lesion Development. Frontiers in Oncology. 2022;12.
98. Staudt MR, Salit J, Kaner RJ, Hollmann C, Crystal RG. Altered lung biology of healthy never smokers following acute inhalation of E-cigarettes. Respiratory Research. 2018;19(1) (no pagination).
99. Tan LH, Bahmed K, Lin C-R, Marchetti N, Bolla S, Criner GJ, et al. The cytoprotective role of DJ-1 and p45 NFE2 against human primary alveolar type II cell injury and emphysema. Scientific Reports. 2018;8.
100. Tang X, Li Z, Yu Z, Li J, Zhang J, Wan N, et al. Effect of curcumin on lung epithelial injury and ferroptosis induced by cigarette smoke. Human & Experimental Toxicology. 2021;40(12_SUPPL):S753-S62.
101. Tomchaney M, Contoli M, Mayo J, Baraldo S, Shuaizhi L, Cabel CR, et al. Paradoxical effects of cigarette smoke and COPD on SARS-CoV2 infection and disease. bioRxiv : the preprint server for biology. 2020.
102. Tsutsumi A, Ozaki M, Chubachi S, Irie H, Sato M, Kameyama N, et al. Exposure to Cigarette Smoke Enhances the Sternness of Alveolar Type 2 Cells. American Journal of Respiratory Cell and Molecular Biology. 2020;63(3):293-305.
103. Van der Toorn M, Marescotti D, Johne S, Baumer K, Bornand D, Dulize R, et al. Comparison of the Biological Effects of Long-Term Exposure of Human Bronchial Epithelial Cells to Total Particulate Matter From 3R4F Cigarette Smoke or Aerosol From the Candidate Modified Risk Tobacco Product (cMRTP) THS2.2. International Journal of Toxicology. 2017;36(1):66-7.
104. Veljkovic E, Jiricny J, Menigatti M, Rehrauer H, Han W. Chronic exposure to cigarette smoke condensate in vitro induces epithelial to mesenchymal transition-like changes in human bronchial epithelial cells, BEAS-2B. Toxicology in Vitro. 2011;25(2):446-53.

105. Vivarelli F, Canistro D, Cirillo S, Elias RJ, Granata S, Mussoni M, et al. Unburned Tobacco Cigarette Smoke Alters Rat Ultrastructural Lung Airways and DNA. Nicotine & Tobacco Research. 2021;23(12):2127-34.
106. Voinsky I, Gurwitz D. Smoking andCOVID-19: Similar bronchialACE2andTMPRSS2expression and higherTMPRSS4expression in current versus never smokers. Drug Development Research. 2020;81(8):1073-80.
107. Wang G, Zhao Q, Zhang H, Liang F, Zhang C, Wang J, et al. Degradation of SARS-CoV-2 receptor ACE2 by the E3 ubiquitin ligase Skp2 in lung epithelial cells. Frontiers of Medicine. 2021;15(2):252-63.
108. Wang Q, Wang H, Zhang Y, Xiao W. Activation of uPAR is required for cigarette smoke extract-induced epithelial-mesenchymal transition in lung epithelial cells. Oncology Research. 2014;21(6):295-305.
109. Wang Y, Pereira EFR, Maus ADJ, Ostlie NS, Navaneetham D, Lei S, et al. Human bronchial epithelial and endothelial cells express alpha 7 nicotinic acetylcholine receptors. Molecular Pharmacology. 2001;60(6):1201-9.
110. Wark PAB, Pathinayake PS, Kaiko G, Nichol K, Ali A, Chen L, et al. ACE2 expression is elevated in airway epithelial cells from older and male healthy individuals but reduced in asthma. Respirology. 2021;26(5):442-51.
111. West KA, Brognard J, Clark AS, Linnoila IR, Yang XW, Swain SM, et al. Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. Journal of Clinical Investigation. 2003;111(1):81-90.
112. Wick KD, Fang X, Maishan M, Matsumoto S, Spottiswoode N, Sarma A, et al. Impact of e-cigarette aerosol on primary human alveolar epithelial type 2 cells. American journal of physiology Lung cellular and molecular physiology. 2022;323(2):L152-L64.
113. Wohnhaas CT, Gindele JA, Kiechle T, Shen Y, Leparc GG, Stierstorfer B, et al. Cigarette Smoke Specifically Affects Small Airway Epithelial Cell Populations and Triggers the Expansion of Inflammatory and Squamous Differentiation Associated Basal Cells. International Journal of Molecular Sciences. 2021;22(14).
114. Yang J, Perez EA, Hou C, Zhang P, Van Scoyk M, Winn RA, et al. Identification of the SARS-CoV-2 Entry Receptor ACE2 as a Direct Target for Transcriptional Repression by Miz1. Frontiers in Immunology. 2021;12.
115. Yang J, Zuo WL, Fukui T, Chao IW, Gomi K, Lee B, et al. Smoking-dependent distal-to-proximal repatterning of the adult human small airway epithelium. American Journal of Respiratory and Critical Care Medicine. 2017;196(3):340-52.
116. Yu H, Li Q, Kolosov VP, Perelman JM, Zhou X. Regulation of Cigarette Smoke-Induced Mucin Expression by Neuregulin1 beta/ErbB3 Signalling in Human Airway Epithelial Cells. Basic & Clinical Pharmacology & Toxicology. 2011;109(1):63-72.
117. Yu XL, Jin XR, Wang DX. Effects of cigarette smoking on the function of metabolizing arachidonic acid and angiotensin I in the isolated perfused rat lungs. Journal of Tongji Medical University = Tong ji yi ke da xue xue bao. 1992;12(4):201-4.
118. Yue X, Basting TM, Flanagan TW, Xu J, Lobell TD, Gilpin NW, et al. Nicotine downregulates the compensatory angiotensin-converting enzyme 2/angiotensin type 2 receptor of the renin–angiotensin system. Annals of the American Thoracic Society. 2018;15(Supplement 2):S126-S7.
119. Zhang L, Gallup M, Zlock L, Basbaum C, Finkbeiner WE, McNamara NA. Cigarette smoke disrupts the integrity of airway adherens junctions through the aberrant interaction of p120-catenin with the cytoplasmic tail of MUC1. Journal of Pathology. 2013;229(1):74-86.
120. Zhang L, Gallup M, Zlock L, Chen YTF, Finkbeiner WE, McNamara NA. Pivotal role of MUC1 glycosylation by cigarette smoke in modulating disruption of airway adherens junctions in vitro. Journal of Pathology. 2014;234(1):60-73.
121. Zhang L, Gallup M, Zlock L, Chen YTF, Finkbeiner WE, McNamara NA. Cigarette Smoke Mediates Nuclear to Cytoplasmic Trafficking of Transcriptional Inhibitor Kaiso through MUC1 and P120-Catenin. American Journal of Pathology. 2016;186(12):3146-59.
122. Zhang L, Gallup M, Zlock L, Finkbeiner WE, McNamara NA. Rac1 and Cdc42 Differentially Modulate Cigarette Smoke Induced Airway Cell Migration through p120-Catenin-Dependent and-Independent Pathways. American Journal of Pathology. 2013;182(6):1986-95.
123. Zhang Y, Huang W, Zheng Z, Wang W, Yuan Y, Hong Q, et al. Cigarette smoke-inactivated SIRT1 promotes autophagy-dependent senescence of alveolar epithelial type 2 cells to induce pulmonary fibrosis. Free Radical Biology and Medicine. 2021;166:116-27.
124. Zhao Y, Xu Y, Li Y, Xu W, Luo F, Wang B, et al. NF-kappaB-mediated inflammation leading to EMT via miR-200c is involved in cell transformation induced by cigarette smoke extract. Toxicological Sciences. 2013;135(2):265-76.
125. Zia S, Ndoye A, Nguyen VT, Grando SA. Nicotine enhances expression of the alpha 3, alpha 4, alpha 5, and alpha 7 nicotinic receptors modulating calcium metabolism and regulating adhesion and motility of respiratory epithelial cells. Research Communications in Molecular Pathology and Pharmacology. 1997;97(3):243-62.